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(54) **Title:** USE OF POLYPEPTIDES, OR NUCLEIC ACIDS ENCODING THEM, OF A 2'-5'-OLIGOADENYLATE SYNTHETASE AND/OR RNASEL FOR DIAGNOSIS, PREVENTION OR TREATMENT OF WOUND HEALING, AND THEIR USE FOR IDENTIFYING PHARMACOLOGICALLY ACTIVE SUBSTANCES

(57) **Abstract:** Use of polypeptides, or nucleic acids encoding them, of a 2'-5'-oligoadenylate synthetase and/or RNAseL for the diagnosis and/or prevention and/or treatment in association with wound healing and/or its pathological disorders, particularly venous ulcers and diabetes-associated poorly healing wounds, and their use for identifying pharmacologically active substances.

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Use of polypeptides, or nucleic acids encoding them, of a 2'-5'-oligoadenylate synthetase and/or RNAseL for diagnosis, prevention or treatment of wound healing, and their use for identifying pharmacologically active substances

The invention relates to the use of polypeptides, or nucleic acids encoding them, of a 2'-5'-oligoadenylate synthetase and/or RNAseL for diagnosis, prevention and/or treatment of wound healing and/or its pathological disorders, particularly venous ulcers and diabetes-associated poorly healing wounds, and to their use for identifying pharmacologically active substances.

In general, wounds heal without any therapeutic treatment. However, there are a large number of diseases in which wound healing is pathologically disturbed, for example diabetes mellitus, arterial occlusion diseases, and innervation disturbances. Disturbances in wound healing can lead to delayed wound healing and to chronic wounds. These disturbances can be caused by the nature of the wound (e.g. wounds of large surface area, and deep and mechanically stretched operation wounds, burns, trauma and decubitus) or the medicinal treatment of the patients (for example with corticoids), or by the nature of the disease itself. Thus, 25% of patients suffering from type II diabetes, for example, frequently suffer from chronic ulcers ("diabetic foot"), about half of which require elaborate in-patient treatment and nevertheless heal poorly. The diabetic foot causes more hospital admissions than does any other complication associated with diabetes. The number of these cases in patients suffering from diabetes type I or type II is increasing and represents 2.5% of all hospital admissions. Furthermore, wounds heal less well as the age of the patient increases. An acceleration of the natural wound healing process is also frequently desirable, for example in order to decrease the risk of bacterial infections or the periods for which patients have to lie in bed.

Further disturbances can also occur after the wound has closed. Whereas wounds in fetal skin heal without scar formation, scars, which frequently constitute a serious cosmetic problem, are always formed after injury in the postnatal period. Furthermore, the quality of life of patients with burn wounds of large surface area can be dramatically impaired, especially since scarred skin also lacks skin appendages, such as hair follicles, sweat glands and sebaceous glands. Given a genetic disposition, keloids, which are hypertrophic scars which proliferate into the surrounding skin, can also be formed.

The process of wound healing requires the complex and coordinated actions and interactions of a variety of cell types. The following steps are distinguished in the wound healing process: blood coagulation in the region of the wound, the recruitment of inflammatory cells, reepithelialization, the formation of granulation tissue and matrix remodeling. Little is so far known with regard to the exact pattern in which the participating cell types react during the phases of proliferation, migration, matrix synthesis and contraction, or with regard to the regulation of genes, such as growth factors, receptors and matrix proteins.

Thus, the therapies which have so far been developed to intervene in wound healing disturbances offer little satisfaction. Established forms of therapy are restricted to physical support of wound healing (e.g. dressings, compresses and gels), or to transplantation of skin tissues, cultured skin cells and/or matrix proteins. In recent years, growth factors have been tested for their ability to improve wound healing without, however, improving the conventional therapy in a decisive manner. Furthermore, the diagnosis of wound healing disturbances is based on optical analysis of the skin, which is not particularly meaningful since a deeper understanding of gene regulation during wound healing is lacking.

Surprisingly, it has now been possible to demonstrate that a 2'-5'-oligoadenylate synthetase plays an essential role in wound healing and its pathological disorders, particularly venous ulcers and diabetes-associated poorly healing wounds,, and that polypeptides according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to

SEQ ID No. 10, or the nucleic acids encoding them, of a 2'-5'-oligoadenylate synthetase, and/or its direct effector RNAseL, are therefore suitable for use in diagnosis, prevention and/or treatment of wound healing and/or its pathological disorders particularly venous ulcers and diabetes-associated poorly healing wounds, and for identifying pharmacologically active substances.

A nucleic acid encoding the 2'-5'-oligoadenylate synthetase polypeptide depicted in SEQ ID No. 1, which can be used in accordance with the invention, was isolated from cDNA libraries which were prepared from intact and wounded skin. In this experiment, those cDNAs were selected which occurred at different frequencies in normally healing wounds as compared to wounds which were treated with dexamethasone and which are therefore healing poorly. This selection was effected by means of subtractive hybridization (Diatchenko et al., 1996, Proc. Natl. Acad. Sci. USA 93: 6025-30). Thereby, it was possible to demonstrate that the 2'-5'-oligoadenylate synthetase was significantly more strongly expressed in wounds of animals which had been treated with the glucocorticoid dexamethasone than it was in untreated wounds.

After the primary identification of a gene, it is necessary to confirm the wound healing-specific expression using a quantitative method. This was done with the aid of TaqMan analysis. These methods were used to determine the quantity of 2'-5'-oligoadenylate synthetase (2-5 OAS) mRNA in tissues from various wound healing states (examples 2 to5). Thereby, it was possible to demonstrate that its expression is regulated differentially in wound healing. Furthermore, it was possible to show that there was a lack of 2-5 OAS mRNA in venous ulcers which lack was even more pronounced in diabetes-associated poorly healing wounds. Both venous ulcers and diabetes-associated poorly healing wounds constitute severe disturbances in wound healing. This demonstrates that disregulation of the expression and/or activity of a 2-5 OAS and/or of its effector can lead to serious wound healing disturbances, particularly venous ulcers and diabetes-associated poorly healing wounds.

According to the invention, the problem is therefore solved by using one or more 2'-5'-oligoadenylate synthetase according to one of SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10, and/or RNAseL polypeptides according to one of SEQ ID No. 11 to SEQ ID No. 12, or functional variants thereof, and/or nucleic acids encoding them, or variants thereof, or a cell which is expressing a 2'-5'-oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10 and/or RNAseL polypeptide according to SEQ ID No. 11 to SEQ ID No. 12 or functional variants thereof and/or nucleic acids encoding them, for diagnosis, treatment and/or prevention of wound healing and/or its pathological disorders, particularly venous ulcers and diabetes-associated poorly healing wounds, and by using them for identifying pharmacologically active substances. Preferred wound healing conditions according to the present invention are mechanically, thermically, chemically, and actinically generated wounds.

Various forms of 2'-5'-oligoadenylate synthetase exist in human cells, namely small, medium-sized and large proteins, with it being possible for the former two proteins in turn to be present in different isoforms as a result of splicing (Reboulliat and Hovanessian, 1999, Journal of Interferon and Cytokine Research, 19: 295-300). The small proteins, i.e. p40 (SEQ ID No. 3) and p46 contain a single catalytic unit and are designated OAS1. They are present as tetramers in vivo. The OAS2 isoforms p67 and p70 (SEQ ID No. 4) contain 2 catalytic units per polypeptide and are present as dimers. In addition, there exists an OAS3 (SEQ ID No. 9) which possesses 3 catalytic units and which is present as a monomer. OAS 1 (SEQ ID No. 1) also exists in the mouse in two splice isoforms. In addition to these polypeptides, whose catalytic activity has been demonstrated, there also exist, both in mice and humans, OAS-like proteins (SEQ ID No. 2 and SEQ ID No. 10) which are characterized by interferon inducibility and homology with the catalytic unit.

2'-5'-Oligoadenylate synthetase and its effector RNAseL are central enzymes of the so-called 2-5A system, which has for a long time been known to be a part of

an antiviral system in living organisms (e.g. Williams, 1979, Nature; 282: 582-586; Chebath, 1987, Nature, 330: 587-588). The quantity of 2'-5'-oligoadenylate synthetase which is present can be induced by interferons (e.g. Baglioni et al., 1979, Biochemistry, 18: 1765-1770). dsRNA or ssRNA with a particular secondary structure, which is found, for example, in the 5'-untranslated region of the HIV virus, is additionally required for activation in vitro (Jacobs and Langland, 1996, Virology, 219: 339-349; Maitra et al., 1994, Virology; 204: 823-827). Following activation, ATP is converted into 2'-5'-linked oligoadenylates of the general formula pppA(2'-5'A)n, $n \geq 2$, which are grouped under the term 2-5A. 2-5A then directly activates the effector RNAseL, which is an endoribonuclease which, after being activated, degrades RNA. This is the basis of the antiviral effect of this enzyme: the presence of genomic viral dsRNA molecules or dsRNA intermediates during the replication of viral genomes leads to the activation of 2-5 OAS, and consequently to the production of 2-5A, to the activation of RNAseL and, finally, to the destruction of the virus or to the inhibition of its replication (Rebouillat and Hovanessian, 1999, Journal of Interferon and Cytokine Research, 19: 295-300). This has been demonstrated, for example, for the case of the EMCV virus (Williams et al., 1979, see above). In addition to the antiviral effect, the 2-5 OAS/RNAseL enzyme system exerts an influence on cell proliferation and differentiation (Hovanessian and Wood, 1980, Virology, 101: 81-90; Rysiecki et al., 1989, J. Interferon Res., 9: 649-657). Thus, various studies suggest 2-5 OAS is important for controlling growth and/or has an antitumorigenic effect (Creasy et al., 1983, Mol. Cell Biol., 3: 780-786; Rimoldi et al., 1990, Exp. Cell Res., 191: 76-82). Furthermore, correlations have been demonstrated to exist between 2-5 OAS and growth competence, proliferation or differentiation in various cell types (Zullo et al., 1985, Cell, 43: 793-800; Maor et al., 1990, Differentiation, 44: 18-24; Birnbaum et al., 1993, Differentiation, 45: 138-145). Apart from viral diseases, the role of 2-5 OAS in diseases in which a relationship with viral diseases is suspected, namely diabetes type I and chronic fatigue syndrome, has also been investigated. It has in fact been possible to demonstrate a regulation in association with these diseases (Bonnevie-Nielsen et al., 1991, J. Interferon Res., 11: 255-260; Suhadolnik et al., 1994, In vivo, 8: 599-604): Lymphocytes form

type I diabetic patients display a significant increased level of 2-5 OAS. However, no clear and convincing evidence exists with regard to the pathogenesis of diabetes II. In addition, a marked up-regulation of the 2-5 OAS/RNAseL system has been demonstrated in patients suffering from chronic fatigue syndrome as well as in lesional skin of psoriatic patients (Schmid et al., 1994, J Interferon Res. 14: 229- 234). It was discussed that the up-regulation of 2-5 OAS in psoriatic lesions is the consequence of the activation of the interferon system. ,. However, no association between 2-5 OAS and/or its effector RNase L and wound healing or its pathological disorders, particularly venous ulcers and diabetes-associated poorly healing wounds, has so far been demonstrated or suggested.

In general, analysis of differentially expressed genes in tissues is subject to markedly more errors, such as falsely positive clones, than does analysis of cell culture systems. This problem cannot be circumvented by using a defined cell culture system since available, simple cell culture systems are unable to adequately simulate the complexity of the wound healing processes in the tissue.

The problem exists, in particular, in the case of the skin, which consists of a large number of different cell types. In addition, the process of wound healing is extremely complicated and comprises temporal and spatial changes in cellular events, such as proliferation and differentiation, in the different cell types. The approach of investigating not only the complex cell system of the skin, in addition to that, the physiological process of wound healing, and even a very wide variety of wound healing stages at the level of differentially expressed genes is therefore not a promising strategy for a skilled person. Because of these difficulties, the success of the screening depended to a considerable extent on the choice of the experimental parameters. Whereas the methods employed (e.g. subtracted hybridization) are standard methods, the screening and verification strategy is itself already inventive as such because of the elaborate and inventive choice of parameters. For example, the time point at which the biopsy is taken is critical for the success of the screening: wound healing disturbances are frequently generated due to disturbances in cell proliferation and cell migration. These processes are

initiated one day after the wounding, for which reason analysis of the molecular processes prior to this point in time would provide little information about the processes which are essential for wound healing which proceeds normally. On the other hand, later than one day after the wounding, the composition of the cell types in the wound changes markedly during the course of wound healing. This can result in a differential expression of a particular gene in the analyzed wound which only occurs due to a difference in cell composition but not due to a change of expression within the single cell. This illustrates the fact that the choice of the day for taking the biopsy had a decisive influence on success of the screening. Despite the parameters which were defined, genes which are differentially expressed during wound healing, but which are unsuitable for use in wound healing were observed to be over-represented. These genes include, for example, genes which encode enzymes of primary metabolism, such as glycolysis, citrate cycle, gluconeogenesis and the respiratory chain, and also genes which encode ribosome proteins, e.g. L41 and S20. It was therefore surprising that the 2'-5'-oligoadenylate synthetase gene, which can be used in accordance with the invention, was identified as being a gene which was relevant to wound healing.

Furthermore, the state of the wound at the time for a possible biopsy of the patient, when the latter first contacts the doctor, varies enormously. An animal model was therefore used for identifying the above-described nucleic acids. BALB/c mice were wounded and wound biopsies were taken at various time points. The advantage of this method is that the limiting conditions, such as genetic background, nature of the wound, time point of the biopsy, etc., can be precisely controlled, thus allowing to analyze gene expression in a reproducible manner. Even under the defined mouse conditions, other problems which impede identification of relevant genes also arise, such as redundancy of the analyzed clones and under-representation of weakly expressed genes.

A wound is characterized by an invasive and complete interruption of the skin barrier with a corresponding loss of substance. The interruption of the skin barrier can be caused by mechanical, thermic, and chemic force as well as by actinic and

ionized radiation. Examples of such wounds are cuts, stab wounds, contused wounds, abrasions, grazes, burns, frostbites, corrosions, and wounds caused by ripping, scratching, pressure, and biting. Normally the different phases of the healing process are induced automatically after wounding. Wound healing disorders develop if the exact coordination of these phases is disrupted. Arteriosclerotic angiopathies, trophic disturbances, caused by peripheral neuropathies or venous insufficiency are triggering factors for these wound healing disorders. Examples of these wound healing disorders are hypertrophic scars, e.g. keloids, ulcers, e.g. arterial ulcers, in particular diabetic and venous ulcers as well as diabetes- associated poorly healing wounds.

In contrast, skin disorders are characterized by changes of skin, such as color changes without any loss of substance, edemas, infiltrations, knots, bladders, scurfs, and pustules. These kind of changes do not lead to a interruption of the skin barrier. However, the skin surface can be enlarged or the tissue can be dilated but not completely interrupted by these changes. The visble changes of the skin occurring during skin disorders are often caused by immunological reaction either against foreign substances or own substances of the organism. The foreign substances (exogenic substances) can contact the organism either systemically or by direct contact with the skin. The inflammation and the visible changes of the skin are reactions against these substances. However, there are also changes of the skin which are caused or modulated by multifactorial inheritance, environmental factors, such as climate, season, infections, allergens, food or emotional factors. The changes of the skin can also be caused by fungi, viruses, leishmanias, parasites or bacteria. Examples of skin disorders are allergies, e.g. urticaria; eczema, e.g. contact eczema; pigmentation disturbances, e.g. vitiligo; pathogen mediated disorders, e.g. rubella, mycosis or erysipelas; psoriasis or atopic dermatitis.

There are also therapeutic differences between wound healing and/or its pathological disorders within the meaning of the invention on one hand and skin disorders on the other hand: Factors causing skin disorders do not automatically

trigger a healing process as it is the case for wound healing. Thus, the therapeutical approach for skin disorders concentrates on combating the triggering factors whereas the therapeutical approaches for wound healing concentrate on triggering the regenerative process or by administrating missing factors. Examples for pharmaceuticals for the treatment of inflammatory skin disorders are corticosteroids and antihistamines. Examples of pharmaceuticals employed in the treatment of pathogenic skin disorders are antibiotics or antimycotics which make the pathogens disappear. Further pharmaceuticals for systemic treatment of skin disorders comprise drugs inducing keratinolysis, or those stopping itching and proliferation.

In contrast, therapeuticals for wound healing and/or its pathological disorders comprise physical support of the healing process, such as debridement, dressings, compresses, gels, sutures; trophic factors which improve the migration of cells which are relevant to the healing process, e.g. PDGF or KGF- 2; hemaostatic pharmaceuticals or transplantations of skin tissue.

The diseases of wound healing and its pathological disorders within the meaning of the invention, particularly venous ulcers and diabetes-associated poorly healing wounds, within the meaning of the invention are also to be distinguished from skin disorders which are associated with degenerate cell development and cell differentiation, and in particular from skin cancer. In the latter disease, individual cells are transformed, resulting in uncontrolled and autonomous proliferation, i.e. independent from interactions with other cell types, and, thereby pass on the pathological changes to their daughter cells. It is therefore a disease which is associated with a loss of interactions, for example of cell-cell adhesion, and of typical cell properties. By contrast, wound healing disorders within the meaning of the invention are due to disturbances of skin cells in their physiological context. The origin of wound healing disorders within the meaning of the invention is determined by a large number of factors. The course of wound healing can be modulated by a very wide variety of endogenous and exogenous factors. Even small disturbances in the interaction between the different cell types of the dermis

and epidermis itself, and also interaction with other tissues and organs, such as the vascular system, the nervous system and the connective tissue, can lead to disturbed wound healing followed by hypertrophic scar formation. Furthermore, infections, aging, diseases such as diabetes and immune diseases, and also vitamin deficiencies, can impair the wound healing process.

The autonomous character of cancer diseases can also be seen at the therapeutic level. In the case of non-metastasizing tumors, cancer can be treated surgically. This possibility of physical treatment exists because no interactions take place between the tumor cells and the surrounding cells and tissues so that the patient can usually be cured by simply excising the tumor; by contrast, this is not possible in the case of wound healing disorders within the meaning of the invention - the pathological disorders in the cell-cell and/or tissue-tissue interactions cannot be remedied by excising affected skin sites. The fact that the diseases which have been compared are diseases which are based on fundamentally different mechanisms becomes clear when the therapeutic approaches are compared as it is the case when comparing wound healing disorders with skin disorders. In the case of cancer diseases and diseases which are associated with degenerate cell proliferation, the therapy is directed toward destroying rapidly growing cells, for example using cytostatic agents. These toxic substances prevent strongly proliferating cells from growing, whereas cells in the G0 phase of cell cycle are unaffected. On the other hand, the treatment of diseases of wound healing within the meaning of the invention is aimed at modulating the interactions between the different cell types, for example by exerting an influence on the migration, proliferation and differentiation of *individual* cell types. Wound healing disorders within the meaning of the invention cannot be cured by a general inactivation of all proliferating cells, irrespective of the cell type. Contrary, one major cause of wound healing disorders is decreased cell proliferation. The methodological approach for identifying the nucleic acids which are used in accordance with the invention and which are involved in wound healing and/or its pathological disorders within the meaning of the invention differs markedly from methods which are suitable for identifying nucleic acids which are involved in the

processes of cancer diseases. The latter can be identified by analyzing differentially expressed genes of the cell type which is affected by cancer. However, by contrast, the aim of the assay of the present invention is to use a comparison of expression in diseased and healthy tissue biopsies to identify genes which are involved in the complex processes of wound healing and/or its pathological disorders, particularly venous ulcers and diabetes-associated poorly healing wounds. This method would be unsuitable for identifying genes which are relevant for cancer.

Within the meaning of the present invention, the term "skin disorder" is to be understood as changes of skin which leaves the skin barrier intact. Thus, these changes can enlarge or dilate the organization of the skin cells but they do not interrupt it completely. Examples of those changes are dermal infiltrates, edema, fluid accumulations or epidermal acanthosis, spongiotic bladders, scurfs or pustules. These changes are non- autonomous with regard to the neighboring tissue.

Within the meaning of the present invention, the term "skin cancer" is to be understood as a malignant, uncontrolled epithelial cell growth within the epidermis or a skin metastasis derived from a different carcinoma elsewhere within the body. Examples of malignant, uncontrolled cell growth are basalioms, basal carcinoma or carcinoma evolving from the plate epithelium. The uncontrolled cell growth is characterized by an exactly defined increase of tissue and is autonomous from the neighboring tissue.

With the meaning of the present invention, the term "wound healing" is to be understood as a regenerative process of the skin after an injury. In contrast to a skin disorder, a wound is characterized by a complete interruption of the skin barrier with loss of substance and cell damage and the subsequent induction of an exact temporal and spatial healing program. Examples of such wounds are mechanical wounds caused by external force, thermic, actinic or chemical means. Wound healing disorders develop if this regenerative process can not be started,

finished or if this regenerative process overshoots. Examples of wounds are cuts, stab wounds, contused wounds, abrasions, grazes, burns, frostbites, corrosions, and wound caused by ripping, scratching, pressure, and biting. Examples of disturbed wound healing are the wounds of diabetic patients and alcoholics, wounds which are infected with microorganisms, ischemic wounds and the wounds of patients suffering from deficient blood supply and venous stasis. Wounds which heal poorly and which are particularly preferred are diabetic, neuropathic, venous, decubitus and arterial ulcers, in particular venous ulcers and diabetes- associated poorly healing wounds.

Within the meaning of the invention "pathological disorders of wound healing" encompass disorders which are characterized by a deficiency of 2'-5'-oligo-adenylate synthetase mRNA, in particular an ulcer of the skin; an especially preferred ulcer is a diabetes- associated ulcer and/or a venous ulcer.

Within the meaning of the present invention, the term "functional variants" of a polypeptide encompasses polypeptides which are regulated, for example like the polypeptides which are used in association with regenerative processes of the skin; in particular, however, in association with disturbances in wound healing, particularly venous ulcers and diabetes-associated poorly healing wounds. Functional variants also include, for example, polypeptides which are encoded by a nucleic acid which is isolated from tissues which are not skin-specific, e.g. embryonic tissue, but which, after expression in a cell which is involved in wound healing , then possess the designated functions.

Within the meaning of the present invention, functional variants are also 2'-5'-oligoadenylate synthetase and RNAseL polypeptides which exhibit a sequence homology, in particular a sequence identity, of approx. at least 70%, preferably approx. at least 80%, in particular approx. at least 90%, especially approx. at least 95%, with the polypeptide having the amino acid sequence depicted in one of SEQ ID No. 1 to SEQ ID No. 4 and SEQ ID No. 9 to SEQ ID No. 12. Examples of such functional variants are therefore the polypeptides which are homologous

with a polypeptide which can be used in accordance with the invention and which are derived from organisms other than the human being or the mouse, preferably from non-human mammals, such as monkeys, pigs and rats. Other examples of functional variants are polypeptides which are encoded by different alleles of the gene, in different individuals or in different organs of an organism. Particularly preferred examples are splicing isoforms of 2'-5'-oligoadenylate synthetase.

Sequence identity is understood as degree of identity (% identity) of two sequences, that in the case of polypeptides can be determined by means of for example BlastP 2.0.1 and in the case of nucleic acids by means of for example BLASTN 2.014, wherein the filter is set off and BLOSUM is 62 (Altschul et al., 1997, Nucleic Acids Res., 25:3389-3402). "Sequence homology" is understood as similarity (% positives) of two polypeptide sequences determined by means of for example BlastP 2.0.1 wherein the filter is set off and BLOSUM is 62 (Altschul et al., 1997, Nucleic Acids Res., 25:3389-3402).

Functional variants of the polypeptide can also be parts of the 2'-5'-oligoadenylate synthetase polypeptide or RNAseL polypeptide which is used in accordance with the invention which have a length of at least 6 amino acids, preferably a length of at least 8 amino acids, in particular a length of at least 12 amino acids. N- and/or C-terminal and/or internal deletions of the polypeptide used in accordance with the invention in the range of approx. 1-60, preferably of approx. 1-30, in particular of approx. 1-15, especially of approx. 1-5, amino acids are also included. For example, the first amino acid, i.e. methionine, can be missing without the function of the polypeptide being significantly altered. Furthermore, a posttranslational modification, for example a myristoylation can be missing without the activity of the enzyme being significantly altered (Samantha et al., 1983, J. Biol. Chem. 255: 9807-9813).

In order to decide, whether a polypeptide is a candidate for a functional variant, the activity of this candidate may be compared with the activity of a polypeptide according to the invention. Assuming that the candidate fulfills the criteria of a

functional variant on the level of % sequence identity the candidate represents a functional variant if the activity in the functional assays is similar to or identical with the activity exhibited by the polypeptide useable according to the invention.

The functional assays for wound healing comprise, for example, the application of an expression vector containing a nucleic acid coding for the candidate polypeptide or the application of the candidate polypeptide itself or of an antibody directed against the candidate polypeptide or of an antisense oligonucleotide to wounds. After incubation of, for example an expression vector, the progress of wound healing of wounds that have been injected with different expression vectors containing either the nucleic acid coding for the candidate functional variant polypeptide the expression vector containing the nucleic acid coding for the polypeptide according to the invention is compared. Such assays may also be applied to test the activity of functional variant polypeptide candidates in the case of disorders of wound healing employing for example badly healing wounds of dexamethasone-treated animals. For example, it was demonstrated that application of the polypeptide-variants PDGF-A and PDGF-B on badly healing rabbit wounds resulted in a comparable wound healing response (J. Surg. Res., 2000, 93:230-236).

The term "encoding nucleic acid" relates to a DNA sequence which encodes an isolated, bioactive 2'-5'-oligoadenylate synthetase and/or RNAseL polypeptide according to the invention or a precursor, for example one possessing a signal sequence. The polypeptide can be encoded by a full-length sequence or any part of the encoding sequence, as long as the specific, for example enzymatic, activity is preserved.

It is known that changes in the sequence of the above-described nucleic acids can be present, for example as a result of the degeneracy of the genetic code, or that non-translated sequences can be included, for example at the 5'- and/or 3'-end of the nucleic acid, without the activity of these nucleic acids being significantly altered. The modifications which are described in more detail below can also be

carried out. This invention therefore also encompasses so-called "variants" of the above-described nucleic acids.

"Variants" of the nucleic acids are to be understood as being all the DNA sequences which are complementary to a DNA sequence which hybridizes, under stringent conditions, to the reference sequence. In addition the polypeptide encoded by the variant exhibits an activity which is essentially the same as that exhibited by the polypeptide encoded by the reference sequence.

The activity of a 2'-5'-oligoadenylate synthetase which can be used in accordance with the invention consists in the dsRNA-dependent conversion of ATP into 2-5A. Assays for determining this activity are summarized in Player and Torrence (1998, Pharmacol. Ther., 78: 55-113).

The activity of an RNAseL which can be used in accordance with the invention consists in the 2-5A-dependent degradation of RNA and in binding 2-5A. Suitable assays are to be found in Player and Torrence (1998, Pharmacol. Ther., 78: 55-113).

"Stringent hybridization conditions" are to be understood as being those conditions which allow a hybridization, for example, at 60°C in 2.5 × SSC buffer, followed by several washing steps at 37°C in a lower buffer concentration, and with the hybridization remaining stable.

Variants of the nucleic acid can also be part of the nucleic acid used in accordance with the invention having a length of at least 8 nucleotides, preferably having a length of at least 18 nucleotides, in particular having a length of at least 24 nucleotides, particularly preferably having at least 30 nucleotides, and most preferably having at least 42 nucleotides.

The term "regulation" is understood, for example, as being an increase or decrease in the quantity of polypeptide or the nucleic acid encoding it, with it being

possible for this change to take place, for example, on a transcriptional or translational or posttranslational level.

The nucleic acids which can be used in accordance with the invention are preferably DNA or RNA, preferably a DNA, in particular a double-stranded DNA. Furthermore, the sequence of the nucleic acids can be characterized by the fact that it possesses at least one intron and/or a polyA sequence. The nucleic acids which are used in accordance with the invention can also be present in the form of their antisense sequence.

In general, a double-stranded DNA is preferred for the expression of the relevant 2'-5'-oligoadenylate synthetase or RNAseL gene, with particular preference being given to the DNA region which encodes the polypeptide. In the case of eukaryotes, this region begins with the first start codon (ATG) located in a Kozak sequence (Kozak, 1987, Nucleic Acids Res. 15: 8125-48) and continues to the next stop codon (TAG, TGA or TAA) which is located in the same reading frame as the ATG. In the case of prokaryotes this region begins with the first AUG (or GUG) after a Shine-Dalgarno sequence and ends with the next stop codon (TAA, TAG or TGA), which lies in the same reading frame to the ATG.

In another embodiment of the present invention a nucleic acid sequences useable according to the invention is used for generating antisense oligonucleotides (Zheng and Kemeny, 1995, Clin. Exp. Immunol. 100: 380-2; Nellen and Lichtenstein, 1993, Trends Biochem. Sci. 18: 419-23; Stein, 1992, Leukemia 6: 967-74) and/or ribozymes (Amarzguioui, et al., 1998, Cell. Mol. Life Sci. 54: 1175-202; Vaish, et al., 1998, Nucleic Acids Res. 26: 5237-42; Persidis, 1997, Nat. Biotechnol. 15: 921-2; Couture and Stinchcomb, 1996, Trends Genet. 12: 510-5). Antisense oligonucleotides can be used to decrease the stability of nucleic acids and/or inhibit the translation of nucleic acids. Thus, the nucleic acid sequences can be used, in accordance with the invention, to decrease, for example, expression of the corresponding genes in cells both *in vivo* and *in vitro*. Antisense oligonucleotides and ribozymes can therefore be suitable for establishing suitable

assays to find pharmaceutical active substances and/or diagnostics. This strategy is also suitable, for example, for skin and epidermal and dermal cells, in particular, when the antisense oligonucleotides are complexed with liposomes (Smyth et al., 1997, J. Invest. Dermatol. 108: 523-6; White et al., 1999, J. Invest. Dermatol. 112: 699-705; White et al., 1999, J. Invest. Dermatol. 112: 887-92). A single-stranded DNA or RNA is preferred for use as a probe or as an antisense oligonucleotide.

It is furthermore possible to use a nucleic acid which has been prepared synthetically for implementing the invention. Thus, the nucleic acid which is used in accordance with the invention can be synthesized chemically, for example in accordance with the phosphotriester method, with the aid of the DNA sequences which are described in table 1 and/or with the aid of the protein sequences which are likewise described in table 1 by referring to the genetic code (see, for example, Uhlmann, E. & Peyman, A. (1990) Chemical Reviews, 90, 543-584, No. 4).

Usually, oligonucleotides are rapidly degraded by endonucleases or exonucleases, in particular by DNases and RNases which are present in the cell. It is therefore advantageous to modify a nucleic acid in order to stabilize it against degradation in a way that high concentrations of the nucleic acid are maintained in the cell over a long period (Beigelman et al., 1995, Nucleic Acids Res. 23: 3989-94; Dudycz, 1995, WO 95/11910; Macadam et al., 1998, WO 98/37240; Reese et al., 1997, WO 97/29116). Such a stabilization can typically be obtained by inserting one or more internucleotide phosphorus groups or by inserting one or more non-phosphorus internucleotides.

Suitable modified internucleotides are summarized in Uhlmann and Peymann (1990 Chem. Rev. 90, 544) (see also Beigelman et al., 1995 Nucleic Acids Res. 23: 3989-94; Dudycz, 1995, WO 95/11910; Macadam et al., 1998, WO 98/37240; Reese et al., 1997, WO 97/29116). Modified internucleotide phosphate radicals and/or non-phosphorus bridges in a nucleic acid useable according to the invention comprise, for example, methylphosphonate, phosphorothioate,

phosphoramidate, phosphorodithioate or phosphate ester, whereas non-phosphorus internucleotide analogs contain, for example, siloxane bridges, carbonate bridges, carboxymethyl esters, acetamidate bridges and/or thioether bridges. It is also intended that this modification should improve the life time of a pharmaceutical composition which can be employed in one of the uses according to the invention.

In another embodiment of the use according to the invention, the above-described nucleic acids are contained in a vector, preferably in shuttle vector, a phagemid, a cosmid, an expression vector or a vector which is applicable in gene therapy. In addition, the above-described nucleic acids can be present in knock-out gene constructs or expression cassettes.

A vector which is applicable in gene therapy preferably contains wound-specific regulatory sequences which are functionally linked to the above-described nucleic acid.

The expression vectors can be prokaryotic or eukaryotic expression vectors. Examples of prokaryotic expression vectors are the pGEM vectors or pUC derivatives, for expression in *E.coli*, whereas examples of eukaryotic expression vectors are the vectors p426Met25 and p426GAL1 (Mumberg et al. (1994) Nucl. Acids Res., 22, 5767-5768) for expression in *Saccharomyces cerevisiae*, *baculovirus* vectors, as disclosed in EP-B1-0 127 839 or EP-B1-0 549 721, for expression in insect cells, and the vectors Rc/CMV and Rc/RSV, or SV40 vectors, for expression in mammalian cells, all of which vectors are generally available.

In general, the expression vectors also contain promoters which are suitable for the corresponding cell, such as the trp promoter for expression in *E.coli* (see, e.g., EP-B1-0 154 133), the Met 25, GAL 1 or ADH2 promoter for expression in yeasts (Russel et al. (1983), J. Biol. Chem. 258, 2674-2682; Mumberg, see above), and the baculovirus polyhedrin promoter for expression in insect cells (see e.g. EP-B1-0 127 839). Promoters which permit constitutive, regulatable, tissue-

specific, cell cycle-specific or metabolism-specific expression in eukaryotic cells are suitable, for example, for expression in mammalian cells. Regulative elements in accordance with the present invention are promoters, activator sequences, enhancers, silencers and/or repressor sequences.

The expression of genes which are relevant to wound healing preferably takes place under the control of tissue-specific promoters, with skin-specific promoters, such as the human K10 promoter (Bailleul et al., 1990. Cell 62: 697-708), the human K14 promoter (Vassar et al., 1989, Proc. Natl. Acad. Sci. USA 86: 1563-67) or the bovine cytokeratin IV promoter (Fuchs et al., 1988; The biology of wool and hair (eds.: G.E. Rogers, et al.), pp. 287-309. Chapman and Hall, London/New York) being particularly preferred.

Other examples of regulative elements which permit tissue-specific expression in eukaryotes are promoters or activator sequences from promoters or enhancers of those genes which encode proteins which are only expressed in particular cell types.

Examples of regulative elements which permit cell cycle-specific expression in eukaryotes are promoters of the following genes: cdc25A, cdc25B, cdc25C, cyclin A, cyclin E, cdc2, E2F-1 to E2F-5, B-myb or DHFR (Zwicker J. and Müller R. (1997) Trends Genet. 13, 3-6). The use of cell cycle-regulated promoters is particularly preferred in cases in which expression of the polypeptides or nucleic acids used in accordance with the invention is to be restricted to proliferating cells.

Examples of suitable regulative elements which permit constitutive expression in eukaryotes are promoters which are recognized by RNA polymerase III or viral promoters, CMV enhancer, CMV promoter, SV40 promoter or LTR promoters, e.g. derived from MMTV (mouse mammary tumor virus; Lee et al. (1981) Nature 214, 228-232) and other viral promoter and activator sequences which are derived from, for example, HBV, HCV, HSV, HPV, EBV, HTLV or HIV.

Examples of regulative elements which permit temporal regulative expression in eukaryotes are the tetracycline operator in combination with an appropriate repressor (Gossen M. et al. (1994) Curr. Opin. Biotechnol. 5, 516-20).

An example of a regulative element which permit simultaneously temporal and spatial limited expression are nucleic acids which encode a fusion between the sequence encoding for the site specific recombinase Cre and a modified estrogen receptor under the control of a tissue specific promoter. The resulting, tissue-specific cytoplasmic fusion protein can be translocated into the nucleus upon administration of the estrogen analog tamoxifen. Upon translocation into the nucleus, the fusion protein causes a site specific recombination which lead to a change in gene expression (Feil et al., 1996, Proc Natl Acad Sci 93: 10887-90).

An example of a regulative element which permits keratinocyte-specific expression in skin is the FiRE element (Jaakkola et al., 2000, Gen. Ther., 7: 1640-1647). The FiRE element is an AP-1-driven, FGF-inducible response element of the syndecan-1 gene (Jaakkola et al., 1998, FASEB J., 12: 959-9).

Examples of regulative elements which permit metabolism-specific expression in eukaryotes are promoters which are regulated by hypoxia, by glucose deficiency, by phosphate concentration or by heat shock.

An example of a regulative element which permits skin-specific expression is the FiRE element.

In order to enable the above-described nucleic acids to be introduced into a eukaryotic or prokaryotic cell by means of transfection, transformation or infection of the nucleic acid can be present as a plasmid, or as a part of a viral or non-viral vector. After introduction into the cell, the nucleic acids will be translated to the polypeptide. Particularly suitable viral vectors are: baculoviruses, vacciniaviruses, adenoviruses, adeno-associated viruses and herpesviruses.

Particularly suitable non-viral vectors are: virosomes, liposomes, cationic lipids and polylysine-conjugated DNA.

Examples of vectors which are applicable in gene therapy are viral vectors, for example adenoviral vectors or retroviral vectors (Lindemann et al., 1997, Mol. Med. 3: 466-76; Springer et al., 1998, Mol. Cell. 2: 549-58). Eukaryotic expression vectors are suitable for use in gene therapy when present in isolated form since naked DNA can penetrate into skin cells when applied topically (Hengge et al., 1996, J. Clin. Invest. 97: 2911-6; Yu et al., 1999, J. Invest. Dermatol. 112: 370-5).

Vectors which are applicable in gene therapy can also be obtained by complexing the above-described nucleic acid with liposomes, since this makes it possible to achieve a very high efficiency of transfection, particularly of skin cells (Alexander and Akhurst, 1995, Hum. Mol. Genet. 4: 2279-85). In lipofection, small, unilamellar vesicles consisting of cationic lipids are prepared by subjecting the liposome suspension to ultrasonication. The DNA is ionically bound on the surface of the liposomes, in a way that a positive net charge remains and 100% of the plasmid DNA is complexed by the liposomes. In addition to the DOTMA (1,2-dioleyloxypropyl-3-trimethylammonium bromide) and DPOE (dioleoylphosphatidylethanolamine) lipid mixtures employed by Felgner et al. (1987, see above), a large number of new lipid formulations are now available and tested for their efficiency in the transfection of various cell lines (Behr et al. (1989), Proc. Natl. Acad. Sci. USA 86, 6982-6986; Gao and Huang (1991), Biochim. Biophys. Acta 1189, 195-203; Felgner et al. (1994) J. Biol. Chem. 269, 2550-2561). Examples of the new lipid formulations are DOTAP N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium ethyl sulfate or DOGS (TRANSFECTAM; dioctadecylamidoglycylspermine). The Cytofectin GS 2888 cationic lipids have also proved to be very well suited for transfecting keratinocytes in vitro and in vivo (US 5,777,153; Lewis et al., 1996, Proc. Natl. Acad. Sci. USA, 93: 3176-3181). Auxiliaries which increase the transfer of nucleic acids into the cell can be proteins or peptides which are bonded to DNA or

synthetic peptide-DNA molecules, for example, which make it possible to transport the nucleic acid into the nucleus of the cell (Schwartz et al. (1999) Gene Therapy 6, 282; Brandén et al. (1999) Nature Biotech. 17, 784). Auxiliaries also encompass molecules which enable nucleic acids to be released into the cytoplasm of the cell (Planck et al. (1994) J. Biol. Chem. 269, 12918; Kichler et al. (1997) Bioconj. Chem. 8, 213) or liposomes, for example (Uhlmann and Peymann (1990) see above). Another particularly suitable form of vector for gene therapy can be obtained by binding the above-described nucleic acid to gold particles and using a Gene Gun to shoot the particles into tissue, preferably the skin, or cells (Wang et al., 1999, J. Invest. Dermatol., 112: 775-81, Tuting et al., 1998, J. Invest. Dermatol. 111: 183-8).

Another embodiment of a vector which is applicable in gene therapy and which can be used in accordance with the invention can be prepared by introducing "naked" expression vectors into a biocompatible matrix, for example a collagen matrix. This matrix can be introduced into wounds in order to transfect the migrating cells with the expression vector and thereby expressing the polypeptides according to the invention in the cells (Goldstein and Banadio, US 5,962,427).

For the genetherapeutic use of the above-described nucleic acid, it is advantageous that the part of the nucleic acid which encodes the polypeptide contains one or more non-coding sequences, including intron sequences, preferably between the promoter and the start codon of the polypeptide and/or a polyA sequence, in particular the naturally occurring polyA sequence or an SV40 virus polyA sequence, especially at the 3' end of the gene. This will stabilize the mRNA (Palmiter et al., 1991, Proc. Natl. Acad. Sci. USA 88: 478-482; Jackson, 1993, Cell 74: 9-14).

Knock-out gene constructs are known to the skilled person from US Patents 5,625,122; US 5,698,765; US 5,583,278 and US 5,750,825, for example.

Another preferred embodiment of the present invention is the use of a cell, preferably an autologous or a heterologous cell, in particular a skin cell, which is transformed with a vector which can be used in accordance with the invention or a knock-out gene construct, for diagnosis and/or prevention and/or treatment of wound healing and/or its pathological disorders, particularly venous ulcers and diabetes- associated poorly healing wounds and for identifying pharmacologically active substances. Cells can be either prokaryotic or eukaryotic cells; examples of prokaryotic cells are *E.coli*, and examples of eukaryotic cells are *Saccharomyces cerevisiae* or insect cells. Examples of skin cells are keratinocytes, fibroblasts and endothelial cells.

A preferred transformed cell which can be used in accordance with the invention is a transgenic, embryonic, non-human stem cell which is characterized by at least one knock-out gene construct which can be used in accordance with the invention and/or at least one expression cassette which can be used in accordance with the invention, as described above. Methods for transforming cells and/or stem cells are well known to the skilled person and include, for example, electroporation and microinjection.

A particularly preferred transformed cell is a skin cell, such as a keratinocyte, a fibroblast, and/or an endothelial cell which expresses a nucleic acid or a protein according to the present invention.

Transgenic, non-human mammals, whose genome contains at least one knock-out gene construct which can be used in accordance with the invention and/or at least one expression cassette which can be used in accordance with the invention, as previously described can be used for diagnosis and/or prevention and/or treating of wound healing and/or its pathological disorders, particularly venous ulcers and diabetes- associated poorly healing wounds, or for identifying pharmacologically active substances. Depending on the promoter employed, transgenic animals which contain one of the above-described expression cassettes generally exhibit an expression of the nucleic acids and/or polypeptides which is increased in a

tissue-specific manner and can be used for analyzing disturbances in wound healing. Thus, an activin A transgenic mouse, for example, exhibits improved wound healing (Munz et al., 1999, EMBO J. 18: 5205-15) whereas a transgenic mouse possessing a dominant negative KGF receptor exhibits delayed wound healing (Werner et al., 1994, Science 266: 819-22). In addition, previously described transgenic animals can be provided with improved wound healing properties.

Methods for preparing transgenic animals, in particular transgenic mice, are likewise known to the skilled person from DE 196 25 049 and US 4,736,866; US 5,625,122; US 5,698,765; US 5,583,278 and US 5,750,825, and comprise transgenic animals which can be generated, for example, by way of the direct injection of expression vectors (see above) into embryos or spermatocytes or by way of the transfection of expression vectors into embryonic stem cells (Polites and Pinkert: DNA Microinjection and Transgenic Animal Production, pages 15 to 68 in Pinkert, 1994: Transgenic Animal Technology: A Laboratory Handbook, Academic Press, London, UK; Houdebine, 1997, Harwood Academic Publishers, Amsterdam, the Netherlands; Doetschman: Gene Transfer in Embryonic Stem Cells, pages 115 to 146 in Pinkert, 1994, see above; Wood: Retrovirus-Mediated Gene Transfer, pages 147 to 176 in Pinkert, 1994, see above; Monastersky: Gene Transfer Technology: Alternative Techniques and Applications, pages 177 to 220 in Pinkert, 1994, see above).

If the previously described nucleic acids, which can be used in accordance with the invention, are integrated into so-called targeting vectors or gene targeting constructs (Pinkert, 1994, see above), it is possible, following transfection of embryonic stem cells and homologous recombination, to generate, for example, gene deficient mice which, in general, as heterozygous mice, exhibit reduced expression of the nucleic acid, whereas homozygous mice no longer exhibit any expression of the nucleic acid. The animals which have been generated in this way can also be used for analyzing wound healing disorders, particularly venous ulcers and diabetes- associated poorly healing wounds. Thus, the eNOS- (Lee et al.,

1999, Am. J. Physiol. 277: H1600-H1608), Nf-1 (Atit et al., 1999, J. Invest. Dermatol. 112: 835-42) and osteopontin- (Liaw et al., 1998, J. Clin. Invest. 101: 967-71) deficient mice, for example, exhibit delayed wound healing. In addition, a tissue-specific reduction in the expression of genes which are relevant to wound healing, for example in skin-specific cells, as achieved using the Cre-loxP system (stat3-deficient mouse, Sano et al., EMBO J. 1999 18: 4657-68) is to be preferred, particularly a concomitant spatial and temporal deletion using the Cre-ER(T) system (Metzger et al., Methods 2001 1: 71-80). Transgenic and gene-targeted cells or animals which have been generated in this way can also be used for screening and for identifying pharmacologically active substances and/or vectors which are active in gene therapy.

2'-5'-Oligoadenylate synthetase and RNAseL polypeptides which can be used in accordance with the invention can be prepared using well-known recombinant methods. In addition, polypeptides which can be used in accordance with the invention can be isolated from an organism or from tissue or cells and used in accordance with the invention. Thus, it is possible, for example, to purify polypeptides which can be used in accordance with the invention from mammalian tissue, for example from skin, or from body fluids, for example blood, serum, saliva, synovial fluid or wound exudates. Furthermore, it is possible to prepare cell lines from cells which are expressing polypeptides which can be used in accordance with the invention. These cell lines can then be used for isolating polypeptides which can be used in accordance with the invention (summarized in Player and Torrence, 998, Pharmacol. Ther., 78: 55-113). For example, expression vectors which contain the nucleic acids which can be used in accordance with the invention can be transformed into skin cells, for example HaCaT cells. The expression can, for example, be constitutive or be inducible. The cells or tissue are preferably interferon-treated.

Furthermore, 2'-5'-oligoadenylate synthetase and RNAseL polypeptides which can be used in accordance with the invention can be prepared recombinantly. For example RNAseL has been prepared using the baculovirus system (Dong et al.,

1994, J. Biol. Chem., 269: 14153-14158). 2'-5'-Oligoadenylate synthetase proteins can likewise be prepared recombinantly (e.g. Rebouillat and Hovanessian, see above).

The 2'-5'-oligoadenylate synthetase or RNAseL polypeptide is, for example, prepared by expressing the above-described nucleic acids in a suitable expression system, as already mentioned above, using methods which are well known to the skilled person. Examples of suitable cells are the E. coli strains DHS, HB101 or BL21, the yeast strain *Saccharomyces cerevisiae*, the insect cell line Lepidopteran, e.g. from *Spodoptera frugiperda*, or the animal cells COS, Vero, 293, HaCaT and HeLa, all of which are generally available.

The 2'-5'-oligoadenylate synthetase and RNAseL polypeptides which can be used in accordance with the invention can be additionally characterized by the fact that they can be prepared synthetically. Thus, the entire polypeptide, or parts thereof, can, for example, be synthesized by means of classical synthesis (Merrifield technique). Parts of the polypeptides according to the invention are suitable, in particular, for obtaining antisera which can then be used to search suitable gene expression libraries in order, in this way, to obtain other functional variants of the polypeptide according to the invention. Thus, p69/p71 and OAS3 were isolated using monoclonal antibodies (Hovanessian et al., 1987, EMBO J, 6: 1273-1280; Hovanessian et al., 1988, J. Biol. Chem., 263: 4945-4949).

Another embodiment relates to the use of the 2'-5'-oligoadenylate synthetase and/or RNAseL polypeptides according to the invention, with the polypeptides being employed in the form of a fusion protein. Fusion proteins which can be used in accordance with the invention can be prepared, for example, by expressing nucleic acids which can be used in accordance with the invention in a suitable cell.

The fusion proteins themselves already exhibit the function of a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide of the invention or else are

only functional after the fusion moiety has been eliminated. These fusion proteins include, in particular, fusion proteins having a content of approx. 1-300, preferably approx. 1-200, in particular approx. 1-100, especially approx. 1-50, foreign amino acids. Examples of such peptide sequences are prokaryotic peptide sequences which can be derived, for example, from *E. coli* galactosidase. Furthermore, it is also possible to use viral peptide sequences, as derived, for example, from the bacteriophage M13, in order to generate fusion proteins for the phage-display method, which is known to the skilled person.

Other preferred examples of peptide sequences for fusion proteins which can be used in accordance with the invention are peptides which facilitate detection of the fusion proteins; these include, for example, green fluorescent protein (WO 95/07463) or functional variants thereof.

In order to purify the above-described 2'-5'-oligoadenylate synthetase and RNAseL polypeptides, an additional polypeptide ("tag") can be added. Protein tags in accordance with the invention permit, for example, high-affinity absorption to a matrix, stringent washing with suitable buffers without eluting the complex to any significant extent, and, subsequently, specific elution of the absorbed complex. Examples of the protein tags which are known to the skilled person comprise a $(His)_6$ tag, a Myc tag, a FLAG tag, a hemagglutinin tag, a glutathione transferase (GST) tag, intein possessing an affinity chitin-binding tag, or a maltose-binding protein (MBP) tag. These protein tags can be located N-terminally, C-terminally and/or internally.

Another embodiment of the invention relates to the use of an antibody or antibody fragment, preferably a polyclonal or monoclonal antibody or antibody fragment, for analyzing, diagnosis, prevention and/or treating of wound healing and/or its pathological disorders, particularly venous ulcers and diabetes- associated poorly healing wounds, and also to its use for identifying pharmacologically active substances, wherein an antibody-producing organism is immunized with a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in

accordance with the invention. Polyclonal and monoclonal antibodies directed against 2'-5'-oligoadenylate synthetase polypeptides which can be used in accordance with the invention are known (Hovanessian et al., 1987, see above; Chebath et al. 1987, J. Biol. Chem., 262: 3852-3857; Marie et al., 1989, Biochem. Biophys. Res. Commun., 160: 580-587).

Thus, the local injection of monoclonal antibodies against TGF beta 1, for example, can improve wound healing in an animal model (Ernst et al., 1996, Gut 39: 172-5).

The method for preparing an antibody or antibody fragment, preferably a polyclonal or monoclonal antibody, is performed according to methods which are well known to the skilled person. These methods comprise the immunizing of a mammal, for example a rabbit, with the 2'-5'-oligoadenylate synthetase or RNAseL polypeptide according to the invention, or functional variants thereof, preferably with parts thereof having a length of at least 6 amino acids, preferably having a length of at least 8 amino acids, in particular having a length of at least 12 amino acids, where appropriate in the presence of, e.g., Freund's adjuvant and/or aluminum hydroxide gels (see, e.g., Diamond, B.A. et al. (1981) The New England Journal of Medicine, 1344-1349). The polyclonal antibodies which have been formed in the animal as the result of an immunological reaction can subsequently be readily isolated from the blood using well-known methods and purified by means of column chromatography, for example. Monoclonal antibodies can, for example, be prepared by the known method of Winter & Milstein (Winter, G. & Milstein, C. (1991) Nature, 349, 293-299). As an alternative to the classical antibodies, it is possible, for example, to use so-called "anticalines", which are based on lipocalin (Beste et al., 1999, Proc. Natl. Acad. Sci. USA, 96: 1898-1903). The natural ligand-binding sites of the lipocalins, such as the retinol-binding protein or the bilin-binding protein, can, for example, be altered, using a "combinatorial protein design" kit. The ligand binding sites than bind to selected haptens, for example to the polypeptides which can be used in accordance with the invention (Skerra, 2000, Biochim. Biophys. Acta 1482: 337-

50). Other known "scaffolds" are known to be alternatives to antibodies for molecular recognition (Skerra, J. Mol. Recognit., 2000, 13: 167-187).

The antibody which can be used in accordance with the invention, or the antibody fragment, are directed against a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide according to the invention and react specifically with the 2'-5'-oligoadenylate synthetase or RNAseL polypeptides according to the invention, with the above mentioned parts of the polypeptide either themselves being immunogenic or with it being possible to make them immunogenic, or to increase their immunogenicity, by coupling them to suitable carriers, such as bovine serum albumin. This antibody, which can be used in accordance with the invention, is either polyclonal or monoclonal; it is preferably a monoclonal antibody. According to the present invention, the term antibody or antibody fragment is also understood as meaning recombinantly prepared and optionally modified antibodies or antigen-binding parts thereof, such as chimeric antibodies, humanized antibodies, multifunctional antibodies, bispecific or oligospecific antibodies, single-stranded antibodies, or F(ab) or $F(ab)_2$ fragments (see, e.g., EP-B1-0 368 684, US 4,816,567, US 4,816,397, WO 88/01649, WO 93/06213, WO 98/24884).

The present invention also relates to the use of at least one 2'-5'-oligoadenylate synthetase and/or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or of a cell which is expressing a 2'-5'-oligoadenylate synthetase and/or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or of an antibody or antibody fragment which is directed against a 2'-5'-oligoadenylate synthetase and/or RNAseL polypeptide which can be used in accordance with the invention, where appropriate combined or together with suitable additives and auxiliaries, for preparing a drug for prevention and/or treating wound healing and/or its pathological disorders, particularly venous ulcers and diabetes-associated poorly healing wounds.

The invention furthermore relates to the use of a drug for prevention and/or treating of wound healing and/or its pathological disorders, particularly venous ulcers and diabetes- associated poorly healing wounds, with at least one 2'-5'-oligoadenylate synthetase and/or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a cell which is expressing a 2'-5'-oligoadenylate synthetase and/or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or an antibody or an antibody fragment which is directed against a 2'-5'-oligoadenylate synthetase and/or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, being employed, where appropriate combined or together with suitable additives and auxiliaries.

The therapy of wound healing and/or its pathological disorders, particularly venous ulcers and diabetes- associated poorly healing wounds, can be effected in a conventional manner, for example using dressings, plasters, compresses or gels which contain the drugs according to the invention. Thus, it is possible to administer the drugs containing suitable additives or auxiliaries, such as physiological sodium chloride solution, demineralized water, stabilizers, proteinase inhibitors, gel formulations, such as white vaseline, low-viscosity paraffin and/or yellow wax, etc., topically and locally in order to exert an immediate and direct effect on wound healing. Administration of the drugs according to the invention can furthermore be effected, likewise topically and locally in the region of the wound, in the form of liposome complexes or gold particle complexes, where appropriate. The treatment can also be effected using a transdermal therapeutic system (TTS), which enables the drugs which can be used in accordance with the invention to be released in a temporarily controlled manner. TTS have been disclosed, for example, in EP 0 944 398, EP 0 916 336, EP 0 889 723 and EP 0 852 493. However, the treatment with the drugs which can be used in accordance with the invention can also be effected by way of oral dosage forms, for example tablets or capsules, by way of the mucous membranes,

for example the nose or the oral cavity, or in the form of depots which are implanted under the skin.

One preferred embodiment of a drug according to the present invention comprises the application of an above- described nucleic acid in form of an above- described viral vector or complexed with liposomes or with gold particles topically and locally in the area of the wound.

A drug which contains the described nucleic acid in naked form, or in the form of one of the above-described vectors which are effective in gene therapy, or in a form in which it is complexed with liposomes or gold particles, is especially suitable for use in human gene therapy. The pharmaceutical excipient is, for example, a physiological buffer solution, preferably having a pH of approx. 6.0-8.0, preferably of approx. 6.8-7.8, in particular of approx. 7.4, and/or an osmolarity of approx. 200-400 milliosmol/liter, preferably of approx. 290-310 milliosmol/liter. The pharmaceutical excipient can additionally contain suitable stabilizers, such as nuclease inhibitors, preferably sequestering agents such as EDTA and/or other auxiliaries which are known to the skilled person.

Administration of the above-described nucleic acid, where appropriate in the form of the viral vectors which are described in more detail above or as liposome complexes or gold particle complexes, is normally effected topically and locally in the region of the wound, for example by using a Gene Gun. It is also possible to administer the polypeptide itself, together with suitable additives or auxiliaries, such as physiological sodium chloride solution, demineralized water, stabilizers, proteinase inhibitors, gel formulations, such as white vaseline, low-viscosity paraffin and/or yellow wax, etc., in order to exert an immediate and direct effect on wound healing.

A further preferred embodiment of a drug according to the present invention comprises the application of polypeptide according to the present invention. For topical application the polypeptide can be combined with suitable auxiliaries, such

as physiological NaCl, demineralized water, stabilizers, protease inhibitors, gel formulations, such as white Vaseline, paraffin and/or yellow wax. These compositions can be administered to influence the wound healing immediately.

A further preferred embodiment of a drug according to the present invention comprises a transformed cell, particularly a autologous or allogene skin cell, which expresses a nucleic acid and a polypeptide of the present invention. The transformed cell can be transplanted by means of suitable carrier systems, such as micro carriers, e.g. a dextran matrix (US 5,980,888).

A further preferred embodiment of a drug according to the present invention is the administration of a catalytically active antibody or an antibody fragment, influencing the function of 2-5 OAS and/or RNase L. Examples of such catalytically active antibobies can be found in Tramontano et al., 1986, Science 234: 1566- 70.

The present invention furthermore relates to the use of at least one 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or of a cell which is expressing a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide or a functional variant thereof, or a nucleic acid which is encoding it, or a variant thereof, or of an antibody or an antibody fragment which is directed against a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, where appropriate combined or together with suitable additives and auxiliaries, for preparing a diagnostic agent for diagnosis wound healing and/or its pathological disorders, particularly venous ulcers and diabetes-associated poorly healing wounds.

For example, it is possible, according to the present invention, to use one of the above-described nucleic acids to prepare a diagnostic agent on the basis of the polymerase chain reaction (examples 2 to 5, PCR diagnosis, e.g. in accordance

with EP 0 200 362) or of an RNase protection assay (see, e.g., Sambrook et al., see above, chapter 7, pages 7.71-7.78; Werner et al., 1992, Growth Factors and Receptors: A Practical Approach 175-197; Werner, 1998, Proc. Natl. Acad. Sci. U.S.A. 89: 6896-6900). These tests are based on the specific hybridization of a nucleic acid with its complementary strand, usually the corresponding mRNA or its cDNA. The nucleic acids which can be used in accordance with the invention can also be modified, for example as disclosed in EP 0 063 879. Preferably, such a DNA fragment is labeled with suitable reagents, for example radioactively with α-P^{32}-dCTP, or non-radioactively with biotin or digoxigenin, using well-known methods, and incubated with isolated RNA which has preferably previously been bound to suitable membranes consisting, for example, of nitrocellulose or nylon. When the quantity of RNA investigated is the same from each tissue sample, it is then possible to determine the quantity of mRNA which has been labeled specifically by the probe. Alternatively, the presence of mRNA can also be determined directly in tissue sections by means of in-situ hybridization (see, e.g., Werner et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89: 6896-900).

The present invention furthermore relates to the use of a diagnostic agent for diagnosis of wound healing and/or its pathological disorders, particularly venous ulcers and diabetes- associated poorly healing wounds, with at least one 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or a cell which is expressing a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or an antibody or an antibody fragment which is directed against a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, being employed, where appropriate combined or together with suitable additives and auxiliaries, for diagnosis of wound healing and/or its pathological disorders, particularly venous ulcers and diabetes- associated poorly healing wounds.

It is consequently also possible, with the aid of the diagnostic agent which can be used in accordance with the invention, to specifically measure *in vitro* the strength of the expression of the given gene in a tissue sample in order, for example, to be able to diagnose a wound healing disturbance with certainty (Examples 2 and5). Such a method is particularly suitable for the early prediction of wound healing disturbances, particularly venous ulcers and diabetes- associated poorly healing wounds which are characterized by a decreased expression of 2-5 OAS (Example 5).

A preferred diagnostic agent according to the invention comprises a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention or the immunogenic parts thereof which have been described in more detail above. The polypeptide or the parts thereof, which are preferably bound to a solid phase, for example consisting of nitrocellulose or nylon, can, for example, be brought into contact, *in vitro*, with the body fluid, e.g. wound exudate, to be investigated in order, in this way, to be able to react, for example, with autoimmune antibodies. The antibody-peptide complex can subsequently be detected with the aid of labeled anti-human IgG or anti-human IgM antibodies, for example. The label consists, for example, of an enzyme, such as peroxidase, which catalyzes a color reaction. The presence, and the quantity of autoimmune antibody which is present, can consequently be detected readily and rapidly by means of a color reaction.

Another diagnostic agent which can be used in accordance with the invention, and which is part of the subject matter of the present invention, comprises the antibodies which can be used in accordance with the invention themselves. These antibodies can be used, for example, to investigate a tissue sample readily and rapidly so as to determine whether the polypeptide concerned is present in an increased quantity in order, thereby, to obtain an indication of possible diseases, in particular wound healing disturbances, particularly venous ulcers and diabetes-associated poorly healing wounds. In this case, the antibodies according to the invention are labeled, for example, with an enzyme, as has already been described

above. This thereby makes it possible to detect the specific antibody-peptide complex readily and also rapidly by way of an enzymatic color reaction.

Another diagnostic agent which can be used in accordance with the invention comprises a probe, preferably a DNA probe, and/or primers. This opens up another possibility of isolating the described nucleic acids from a suitable gene library, for example from a wound-specific gene library, using a suitable probe (see, e.g., J. Sambrook et al., 1989, Molecular Cloning. A Laboratory Manual 2nd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, chapter 8, pages 8.1 to 8.81, chapter 9, pages 9.47 to 9.58 and chapter 10, pages 10.1 to 10.67). Preferably, the probes are bound to solid phase and nucleic acids are brought into contact with the probes. Alternatively, nucleic acids to be analyzed are bound to solid phase and probes are brought into contact for diagnosis. In both cases, detection systems for nucleic acids are well known to the skilled person and comprise for example radioactive labeling or digoxigenine-labeling. Preferably, the nucleic acid to be investigated or the probe for diagnosis bound to solid phase are part of an array.

Examples of suitable probes are DNA or RNA fragments having a length of approx. 100-1000 nucleotides, preferably having a length of approx. 200-500 nucleotides, in particular having a length of approx. 300-400 nucleotides, and whose sequence can be derived from the 2'-5'-oligoadenylate synthetase or RNAseL polypeptides according to SEQ ID No. 1 to SEQ ID No. 4 and SEQ ID No. 9 to SEQ ID No. 12 of the sequence listing and/or using the cDNA sequences of the database entries given in table 1 or using the sequence listing in accordance with one of SEQ ID No. 5 to SEQ ID No. 8 and SEQ ID No. 13 to SEQ ID No. 14.

Alternatively, oligonucleotides which are suitable for use as primers for a polymerase chain reaction can be synthesized with the aid of the derived nucleic acid sequences. These primers can then be used to amplify and isolate the previously described nucleic acid, or parts of this nucleic acid, from cDNA, for

example from wound-specific cDNA (examples 2 to 5). Examples of suitable primers are DNA fragments having a length of approx. 10 to 100 nucleotides, preferably having a length of approx. 15 to 50 nucleotides, in particular having a length of 20 to 30 nucleotides, and whose sequence can be derived from the 2'-5'-oligoadenylate synthetase or RNAseL polypeptides according to SEQ ID No. 1 to SEQ ID No. 4 and SEQ ID No. 9 to SEQ ID No. 12 of the sequence listing and/or using the cDNA sequences of the database entries given in table 1 or using the sequence listing in accordance with one of SEQ ID No. 5 to SEQ ID No. 8 and SEQ ID No. 13 to SEQ ID No. 14.

The invention further relates to the use of at least one 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or of a cell which is expressing a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or of an antibody or antibody fragment which is directed against a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, where appropriate combined or together with suitable additives and auxiliaries, for identifying pharmacologically active substances in association with wound healing and/or its pathological disorders, particularly venous ulcers and diabetes- associated poorly healing wounds.

The present invention furthermore relates to the use of at least one 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or of a cell which is expressing a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or of an antibody or an antibody fragment which is directed against a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof,

where appropriate combined or together with suitable additives and auxiliaries, for preparing a test for identifying pharmacologically active substances in the context with wound healing, in particular wound healing disturbances, particularly venous ulcers and diabetes- associated poorly healing wounds.

Within the meaning of the present invention, the term "pharmacologically active substances" is to be understood as meaning all those molecules, compounds and/or compositions and substance mixtures which can interact, under suitable conditions, with the previously described nucleic acids, nucleic acid analogs, polypeptides, antibodies or antibody fragments, where appropriate together with suitable additives and auxiliaries. While possible interactors can be simple chemical organic or inorganic molecules or compounds, they can also comprise nucleic acids, peptides, proteins or complexes thereof. Because of their interaction, the interactors can exert an influence on the function(s) of the nucleic acids, polypeptides or antibodies *in vivo* or *in vitro* . Alternatively the interactors could just bind to the previously described nucleic acids, polypeptides, antibodies or antibody fragments or perform other interactions with them in a covalent or non-covalent manner.

The present invention furthermore relates to the use of a test for identifying pharmacologically active substances associated with wound healing and/or its pathological disorders, particularly venous ulcers and diabetes- associated poorly healing wounds, with at least one 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or a cell which is expressing a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or an antibody or an antibody fragment which is directed against a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, being employed, where appropriate combined or together with suitable additives and auxiliaries.

Another embodiment of the invention relates to the use of at least one polypeptide which can be used in accordance with the invention or a nucleic acid encoding it, or of a cell which is expressing a polypeptide which can be used in accordance with the invention or a nucleic acid encoding it, or of an antibody or an antibody fragment which is directed against a polypeptide which can be used in accordance with the invention, where appropriate combined or together with suitable additives and auxiliaries, for identifying pharmacologically active substances associated with wound healing and/or its pathological disturbances, particularly venous ulcers and diabetes- associated poorly healing wounds, with the pharmacologically active substances exerting an influence on the activity of at least one 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention. For this, a pharmacologically active substance can be brought into contact with the polypeptide which can be used in accordance with the invention and the change in activity can be determined or the activity can be compared with that of untreated polypeptide.

Suitable assays for determining the activity of the 2'-5'-oligoadenylate synthetase and RNAseL polypeptides which can be used in accordance with the invention are known to the skilled person and are summarized in Reboulliat and Hovanessian (1999, see above) and Player and Torrence (1998, see above).

In order to estimate the activity of a 2'-5'-oligoadenylate synthetase, 2-5A synthetase activity can be measured by means of an enzyme assay in which the end product is subsequently determined. This can be done using radioactively labeled ATP. The subsequent analysis of the 2-5A products can then be carried out by means of HPLC, column chromatography, thin layer chromatography, electrophoresis or binding to DEAE paper (Player and Torrence, 1999, see above; p. 69). In addition, 2-5A can be detected using antibodies which recognize 2-5A end products. Furthermore, it is possible to use a coupled assay which contains both a 2'-5'-oligoadenylate synthetase polypeptide and an RNAseL polypeptide. The activity of a 2'-5'-oligoadenylate synthetase polypeptide which has been

modulated by pharmacologically active substances can then be established by measuring the RNAse activity. Another indirect test consists of measuring the inhibition of protein synthesis in a cell-free system due to degradation of the RNA (Player and Torrence, 1999, see above; p. 69). In addition, the pyrophosphate which is released by the conversion of ATP into 2-5A can be determined by means of a spectrophotometric assay.

In order to estimate the activity of the RNAseL, it is possible either to determine 2-5A binding or the RNAse activity. The 2-5A binding can be established, for example, by means of radioaffinity labeling, as described in Floyd-Smith et al. (1982, J. Biol. Chem., 257: 8584-8587) or Nolan-Sorden et al. (1990, Anal. Biochem., 184: 298-304) or by means of competitive binding with radioactive substrates as described, for example, in Johnston and Torrence (1984; in: Interferon: Mechanism of Production and Action, pages 189-298, Elsevier). It is also possible to carry out a nuclease assay. Suitable nuclease assays consist in the gel-electrophoretic analysis of specific or nonspecific RNAse cleavage products (Wreschner et al., 1981, Nucleic Acid Res., 9: 1571-1581; Williams et al., 1981, Methods Enzymol., 79: 199-208), the degradation of radiolabeled poly(U) by the RNAseL on 2-5A cellulose (Silverman, 1985, Anal. Biochem., 144: 450-460) and the determination of acid-precipitable radioactivity (Biglioni et al., 1981, J. Biol. Chem., 256: 3253-3257).

In one embodiment of the present invention, at least one cell which is expressing a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, is used for identifying pharmacologically active substances in association with wound healing and/or its pathological disorders, particularly venous ulcers and diabetes- associated poorly healing wounds.

A suitable system can be prepared, for example, by means of the stable transformation of epidermal or dermal cells with expression vectors which contain selectable marker genes and the described nucleic acids. In this method, the

expression of the described nucleic acids in the cells is altered such that it corresponds to the pathologically disturbed expression in vivo. Antisense oligonucleotides which bind to the described nucleic acid can also be used for this purpose. It is therefore particularly advantageous for these systems to be acquainted with the expression behavior of the genes in association with disturbed regenerative processes, as disclosed in this application. In this way, it is frequently possible to imitate the pathological behavior of the cells in vitro and substances can be sought which once again restore the normal behavior of the cells and which possess a therapeutic potential.

HaCaT cells, which are generally available, and the expression vector (pCMV4 (Anderson et al., 1989, J. Biol. Chem. 264: 8222-9) are, for example, suitable for these test systems which can be used in accordance with the invention. Thus, the above-described nucleic acid can be integrated into the expression vectors either in the sense orientation or in the anti-sense orientation such that the functional concentration of the mRNA of the corresponding genes in the cells is either increased or decreased by hybridization with the antisense RNA. After the transformation, and selection of stable transformants, the cells in culture generally exhibit an altered proliferation behavior, migration behavior and/or differentiation behavior as compared with control cells. This behavior in vitro frequently correlates with the function of the corresponding genes in regenerative processes in the organism (Yu et al., 1997, Arch. Dermatol. Res. 289: 352-9; Mils et al., 1997, Oncogene 14: 15555-61: Charvat et al., 1998, Exp Dermatol 7: 184-90; Werner, 1998, Cytokine Growth Factor Rev. 9: 153-65; Mythily et al., 1999, J. Gen. Virol. 80: 1707-13) and can be detected using tests which are simple and rapidly implementable such that it is possible develop test systems for pharmacologically active substances based on these tests. Thus, the proliferation behavior of cells can be established very rapidly by means, for example, of the incorporation of labeled nucleotides into the DNA of the cells (see, e.g., Savino and Dardenne, 1985, J. Immunol. Methods 85: 221-6; Perros and Weightman, 1991, Cell Prolif. 24: 517-23; de Fries and Mitsuhashi, 1995, J. Clin. Lab. Anal. 9: 89-95) by staining the cells with specific dyes (Schulz et al., 1994, J. Immunol.

Methods 167: 1-13) or by means of immunological methods (Frahm et al., 1998, J. Immunol. Methods 211: 43-50). Migration can be established readily by means of the "migration index" test (Charvat et al., see above) and comparable test systems (Benestad et al., 1987, Cell Tissue Kinet. 20: 109-19; Junger et al., 1993, J. Immunol. Methods 160: 73-9). Examples of suitable differentiation markers are keratin 6, 10 and 14 and also loricrin and involucrin (Rosenthal et al., 1992, J. Invest. Dermatol. 98: 343-50) whose expression can be readily detected, for example, using generally available antibodies.

Another suitable test system which can be used in accordance with the invention is based on identifying interactions by the Two Hybrid System (Fields and Sternglanz, 1994, Trends in Genetics, 10, 286-292; Colas and Brent, 1998 TIBTECH, 16, 355-363). In this test, cells are transformed with expression vectors which express fusion proteins which consist of the polypeptide according to the invention and a DNA-binding domain of a transcription factor such as Gal4 or LexA. The transformed cells also contain a reporter gene whose promoter contains binding sites for the corresponding DNA-binding domain. By means of transforming a further expression vector, which expresses a second fusion protein consisting of a known or unknown polypeptide and an activation domain, for example from Gal4 or herpes simplex virus VP16, the expression of the reporter gene can be greatly increased if the second fusion protein interacts with the polypeptide according to the invention. This increase in expression can be used for identifying new interacting partners, for example by preparing a cDNA library from regenerating tissue for the purpose of constructing the second fusion protein. This test system can also be used for screening substances which inhibit an interaction between the polypeptide according to the invention and an interacting partner. Such substances decrease the expression of the reporter gene in cells which are expressing fusion proteins of the polypeptide according to the invention and the interacting partner (Vidal and Endoh, 1999, Trends in Biotechnology, 17: 374-81). In this way, it is possible to rapidly identify novel pharmacologically active compounds which can be employed for the therapy of disturbances of regenerative processes.

Another test for identifying pharmacologically active substances consists in contacting a cell, which is expressing a 2'-5'-oligoadenylate synthetase or RNAseL polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof with pharmacologically active substances, determining the activity of the 2'-5'-oligoadenylate synthetase or the RNAseL polypeptide, and comparing this activity with that of untreated cells.

Furthermore, a test system can be based on polypeptides which can be used in accordance with the invention, or functional variants thereof, or nucleic acids encoding them, or variants thereof, or cells expressing polypeptides which can be used in accordance with the invention, or functional variants thereof, or nucleic acids encoding them, or variants thereof or antibodies or antibody fragments which are directed against polypeptides which can be used in accordance with the invention, or functional variants thereof, being bound to a solid phase and substances being tested for interaction, for example binding or change in conformation. Suitable systems, such as affinity chromatography and fluorescence spectroscopy, are known to the skilled person.

The solid phase-bound polypeptides which can be used in accordance with the invention, or functional variants thereof, or nucleic acids encoding them, or a variant thereof, or cells expressing polypeptides which can be used in accordance with the invention, or functional variants thereof, or nucleic acids encoding them, or variants thereof or an antibody or an antibody fragment which is directed against a polypeptide which can be used in accordance with the invention, or a functional variant thereof, can also be part of an array. Methods for preparing such arrays using solid phase chemistry and photolabile protecting groups are known, for example, from US 5,744,305. These arrays can also be brought into contact with substances or substance libraries and tested for interaction, for example binding or change in conformation.

Thus, a substance to be tested can, for example, contain a detectable label; for example, the substance can be radioactively labeled, fluorescence-labeled or luminescence-labeled. Furthermore, substances can be coupled to proteins which permit indirect detection, for example by way of enzymatic catalysis using a peroxidase assay having a chromogenic substrate, or by means of binding a detectable antibody. Changes in the conformation of a polypeptide which can be used in accordance with the invention as a result of interaction with a test substance can be detected, for example, by a change in the fluorescence of an endogenous tryptophane residue in the polypeptide.

Pharmacologically active substances of the polypeptides which can be used in accordance with the invention can also be nucleic acids which are isolated by means of selection methods, such as SELEX (see Jayasena, 1999, Clin. Chem. 45: 1628-50; Klug and Famulok, 1994, M. Mol. Biol. Rep. 20: 97-107; Toole et al., 1996, US 5,582,981). In the SELEX method, single-stranded RNA molecules are typically isolated from a large pool by repeatedly amplifying and selecting those molecules which bind with high affinity to a polypeptide which can be used in accordance with the invention (aptamers). Aptamers can also be synthesized in their mirror-image form, for example as an L-ribonucleotide, and then selected (Nolte et al., 1996, Nat. Biotechnol. 14: 1116-9; Klussmann et al., 1996, Nat. Biotechnol. 14: 1112-5). Forms which have been isolated in this way have the advantage that they are not degraded by naturally occurring ribonucleases and therefore possess greater stability.

Pharmacologically active substances of the polypeptides which can be used in accordance with the invention can also be nucleic acid analogs of 2-5A. Thus, several 2-5 derivatives have so far been identified as being antagonists of the 2-5 effect (Lesiak and Torrence, 1986, J. Med. Chem., 29: 1015-1022; Imai et al., 1982, J. Biol. Chem, 257: 12739-12745; Player and Torrence, 1998, see above, p. 80).

The pharmacologically active substances which have been identified with the aid of the test methods which can be used in accordance with the invention can be employed, where appropriate combined or together with suitable additives and auxiliaries, for producing a diagnostic agent or drug for diagnosis, prevention and/or treating wound healing and/or its pathological disorders, particularly venous ulcers and diabetes- associated poorly healing wounds.

Another embodiment of the invention relates to the use of at least one polypeptide which can be used in accordance with the invention, or of a nucleic acid encoding it, or of a cell which is expressing a polypeptide which can be used in accordance with the invention or a nucleic acid encoding it, or of an antibody or an antibody fragment which is directed against a polypeptide which can be used in accordance with the invention, where appropriately combined or together with suitable additives and auxiliaries, for identifying pharmacologically active substances in association with wound healing and/or its disturbances, particularly venous ulcers and diabetes- associated poorly healing wounds, with the pharmacologically active substances exerting an effect on the expression of at least one nucleic acid which can be used in accordance with the invention.

Assays for identifying pharmacological substances which exert an effect on the expression of genes are well known to the skilled person (see, for example, Sivaraja et al., 2001. US 6,183,956).

Thus, cells which express 2'-5'-oligoadenylate synthetase or RNAseL, for example HeLa cells, can be cultured as a test system for analyzing gene expression *in vitro*, where skin cells, in particular keratinocytes, fibroblasts or endothelial cells, are to be preferred. Thus, the human keratinocyte cell line HaCaT, which is generally available, constitutes a possible test system.

Gene expression is analyzed, for example, at the level of the mRNA or the proteins. The quantity of 2'-5'-oligoadenylate synthetase and/or RNAseL mRNA or protein is measured after adding one or more substances to the cell culture and

compared with the corresponding quantity in a control culture. This takes place, for example, with the aid of the hybridization of an antisense probe, which can be used to detect the mRNA of usable 2'-5'-oligoadenylate synthetase and/or RNAseL which is present in the lysate of the cells. The hybridization can be quantified, for example, by means of binding a specific antibody to the mRNA-probe complex (see Stuart and Frank, 1998, US 4,732,847). It is possible to carry out the analysis using a high-throughput method and to analyze a very large number of substances for their suitability for use as modulators of the expression of 2'-5'-oligoadenylate synthetase or RNAseL (Sivaraja et al., 2001, US 6,183,956). The substances which are to be analyzed can be taken from substance libraries (see, e.g., DE19816414, DE19619373) which can contain several thousand substances, which are frequently very heterogeneous. Alternatively, the total RNA or mRNA can first of all be isolated from cells and the absolute quantity, or the relative proportion of the mRNA of utilizable 2'-5'-oligoadenylate synthetase or RNAseL can then be determined, for example using quantitative RT-PCR (see EP 0 200 362; Wittwer et al., 1997, BioTechniques 22: 130-8; Morrison et al., 1998, BioTechniques 24: 954-62) or the RNAse protection assay (see, e.g., Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, Cold Spring Harbor Laboratory Press, New York, chapter 7; EP 0 063 879). Analyzing the quantity of protein in the cell lysate using antibodies which specifically recognize 2'-5'-oligoadenylate synthetase or RNAseL is another possibility. In this case, the quantification can be effected, for example, using an ELISA or a Western blot, which are well known. In order to determine the specificity of the substances for the expression of 2'-5'-oligoadenylate synthetase and/or RNAseL, the influence of the substances on the expression of 2'-5'-oligoadenylate synthetase and/or RNAseL can be compared with their influence on the expression of other genes, for example metabolic genes such as GAPDH. This can be done either in separate analysis or in parallel with the analysis of the 2'-5'-oligoadenylate synthetase and/or RNAseL heterodimer or its individual components.

The invention furthermore relates to the use of at least one polypeptide which can be used in accordance with the invention, or of a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or a cell expressing a polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof or of an antibody or an antibody fragment which is directed against a polypeptide which can be used in accordance with the invention, or a functional variant thereof, where appropriate combined or together with suitable additives and auxiliaries, for preparing an array, which is fixed to a support material, (1) for the production of an array for the analysis in association with wound healing and/or its pathological disorders, and/or (2) for performing diagnosis or analysis in association with wound healing and/or its pathological disorders, particularly venous ulcers and diabetes-associated poorly healing wounds.

Methods for preparing such arrays using solid phase chemistry and photolabile protecting groups have been disclosed, for example, in US 5,744,305.

The present invention furthermore relates to the use of such an array for performing analysis in association with wound healing and/or its pathological disorders, particularly venous ulcers and diabetes- associated poorly healing wounds, with at least one polypeptide which can be used in accordance with the invention, or a functional variant thereof, or a nucleic acid encoding it, or a variant thereof, or a cell expressing a polypeptide which can be used in accordance with the invention, or a functional variant thereof, or nucleic acids encoding them, or variants thereof or an antibody or an antibody fragment which is directed against a polypeptide which can be used in accordance with the invention or a functional variant thereof, being employed, where appropriate combined or together with suitable additives and auxiliaries.

It is also possible, for example, to use DNA chips and/or protein chips, which comprise at least one nucleic acid, at least one polypeptide and/or at least one antibody or antibody fragment or at least one cell, as previously described, for

performing analysis in association with wound healing and/or its pathological disorders, particularly venous ulcers and diabetes- associated poorly healing wounds. DNA chips have been disclosed, for example, in US 5,837,832.

The invention will now be further clarified using the tables and examples which follow but without being restricted thereto.

Description of the tables and sequences:

Table 1: Tabular summary of the 2'-5'-oligoadenylate synthetase and RNAseL polypeptide sequences which can be used in accordance with the invention and their cDNAs and access numbers and/or SEQ ID numbers.

Table 2: Tabular listing of the altered expression of the 2'-5'-oligoadenylate synthetase gene in the wounds of 10-week-old BALB/c mice and in the wounds of young (4-week-old) and old (12-month-old) mice and also in mice suffering from genetic diabetes.

Table 3: TaqMan analysis of the kinetics of the expression of 2'-5'-oligoadenylate synthetase mRNA during wound healing in the mouse.

Table 4: TaqMan analysis of the kinetics of the expression of 2'-5'-oligoadenylate synthetase mRNA during wound healing in humans.

Table 5: Analysis of the expression of 2'-5'-oligoadenylate synthetase mRNA in human ulcer biopsies.

SEQ ID No. 1 to SEQ ID No. 14 show human or mouse 2'-5'-oligoadenylate synthetase and/or RNAseL polypeptide or cDNA sequences which can be used in accordance with the invention.

SEQ ID No. 15 to SEQ ID No. 26 show DNA sequences of oligonucleotides which were used for the experiments of the present invention.

Examples

Example 1: Enriching 2'-5'-oligoadenylate synthetase cDNA by means of subtractive hybridization and identifying 2'-5'-oligoadenylate synthetase as a gene which is relevant for wounds and skin diseases

Standard methods (Chomczynski and Sacchi, 1987, Anal. Biochem. 162: 156-159, Chomczynski and Mackey, 1995, Anal. Biochem. 225: 163-164) were used to isolate total RNA from the intact skin and wound tissue (wounding on the back by cutting with scissors 1 day before taking biopsies) of BALB/c mice. In order to obtain tissue from mice having wounds which heal poorly, BALB/c mice were treated with dexamethasone (0.5 mg of dexamethasone in isotonic salt solution was injected, per kg of body weight, twice daily for 5 days) prior wounding. The RNAs were then transcribed into cDNA using a reverse transcriptase. The cDNA was synthesized using the "SMART PCR cDNA Synthesis Kit", supplied by Clontech Laboratories GmbH, Heidelberg, in accordance with the instructions in the corresponding manual.

Subtractive hybridization (Diatchenko et al., 1996, Proc. Natl. Acad. Sci. Acad. Sci. U.S.A. 93: 6025-30) was carried out in order to identify the cDNAs which occurred at differing frequencies in the cDNA pools. This was done using the "PCR-Select cDNA Subtraction Kit", supplied by Clontech Laboratories GmbH, Heidelberg, in accordance with the instructions in the corresponding manual, with excess oligonucleotides being removed, after the cDNA synthesis, by means of agarose gel electrophoresis. Two cDNA pools which were enriched for wound-

relevant genes were prepared, with one pool being enriched in cDNA fragments which are more strongly expressed in normally healing wounds as compared with poorly healing wounds ("normally healing cDNA pool") and one pool being enriched in cDNA fragments which are more strongly expressed in poorly healing wounds as compared with normally healing wounds ("poorly healing cDNA pool").

In order to identify the genes which were contained in the cDNA pools relevant to wound healing, the presence of the corresponding cDNAs in the pools was analyzed by reverse Northern blotting. In this method, cDNA fragments are fixed on membranes in the form of arrays of many different cDNAs and are hybridized with a complex mixture of radioactively labeled cDNAs (Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, Cold Spring Harbor Laboratory Press, New York, chapter 9, pages 9.47 to 9.58 and chapter 10, pages 10.38 to 10.50; Anderson and Young: Quantitative filter hybridization; in: Nucleic Acids Hybridization, A Practical Approach, 1985, Eds. Hames and Higgins, IRL Press Ltd.; Oxford, chapter 4, pages 73 to 112). Commercially available membranes (Mouse ATLAS Array, Clontech) were used, for example.

In order to prepare suitable hybridization probes, the subtracted cDNA pools were treated with the restriction endonuclease RsaI and purified by agarose gel electrophoresis (Sambrook et al., see above, chapter 6, pages 6.1 to 6.35), in order to remove the cDNA synthesis and amplification primers (see the "PCR-Select cDNA Subtraction Kit" manual from Clontech). The cDNAs were then radioactively labeled using the random-hexamer priming method (Feinberg and Vogelstein, 1983, Anal. Biochem. 132: 6-13) in order to prepare hybridization probes.

The membrane was preincubated for 30 min, at 65°C, in 25 ml of hybridization solution (25 mM sodium phosphate, pH = 7.5, 125 mM NaCl, 7% SDS). The hybridization probe was denatured for 10 min at 100°C and then cooled on ice; approx. 100 CPM (counts per minute) per ml were added to the hybridization

solution and the hybridization was carried out for 16 hours, at 65°C, in a hybridization incubator. Then, the membrane was washed twice for 10 min, at 65°C, with the hybridization solution without probe. The membrane was then washed several times for 10 min each, at 65°C, in washing solution (2.5 mM sodium phosphate, pH = 7.5, 12.5 mM NaCl, 0.7% SDS) until it was no longer possible to detect any activity in the decanted solution. The radioactive signals were evaluated using a Phosphoimager (BioRad, Quantity One®). The cDNAs which gave different signal intensities with the different probes were then selected. A markedly stronger signal intensity was obtained with the hybridization probe from the "poorly healing cDNA pool" than with that from the " normally healing cDNA pool" at the position of the 2'-5'-oligoadenylate synthetase on the membrane.

<u>Example 2: Using TaqMan analysis to verify the pattern of expression of the 2'-5'-oligoadenylate synthetase (2-5 OAS)</u>

TaqMan analysis, in a GeneAmp5700 supplied by Applied Biosystems, was used to verify the differential expression of the 2'-5'-oligoadenylate synthetase mRNA in dexamethasone-treated wounds and to investigate other wound-healing conditions.

In order to obtain tissue from mice with poorly healing wounds, BALB/c mice were treated with dexamethasone (0.5 mg of dexamethasone in isotonic salt solution were injected, per kg of body weight, twice daily for 5 days) before wounding. In order to obtain tissue from young mice and old mice, 1-day wounds from 4-week-old and 12-month-old BALB/c mice were used. In order to obtain wound tissue from diabetic mice, 1-day wounds from 10-week-old C57BL/Ks-*db/db*/Ola mice were used.

The RNA was isolated by homogenizing the biopsies in RNAclean buffer (AGS, Heidelberg) to which a 1/100 volume of 2-mercaptoethanol had been added using

a dispenser. The RNA was then extracted by phenolizing it twice with water-saturated, acid phenol in the presence of 1-bromo-3-chloropropane. An isopropanol precipitation and an ethanol precipitation were then carried out and the RNA was washed with 75% ethanol. After that, the RNA was digested with DNase I. For this, 20 µg of RNA (made up to 50 µl with DEPC-treated water) were incubated, at 37°C for 20 min, with 5.7 µl of transcription buffer (Roche), 1 µl of RNase inhibitor (Roche; 40 U/µl) and 1 µl of DNase I (Roche; 10 U/µl). A further 1 µl of DNase I was then added and the mixture was incubated at 37°C for a further 20 min. The RNA was then phenolized, precipitated with ethanol and washed. All the above-listed steps were carried out using DEPC (diethylpyrocarbonate)-treated solutions and/or liquids for those solutions/liquids did not contain any reactive amino groups. The cDNA was then prepared from the extracted RNA. This was done in the presence of 1 × TaqMan RT-buffer (Applied Biosystems), 5.5 mM $MgCl_2$ (Perkin Elmer), in each case 500 µM of dNTPs (Perkin Elmer), 2.5 µM random hexamers (Perkin Elmer), 1.25 U/µl of MultiScribe Reverse Transcriptase (50 U/µl Perkin Elmer), 0.4 U/µl RNase inhibitor (20 U/µl, Perkin Elmer), 20 µl of RNA (50 ng/µl) and DEPC-treated water (to a volume of 100 µl). After addition of the RNA, and after thoroughly mixing, the solution was aliquoted into 2 × 0.2 ml tubes (50 µl in each case) and the reverse transcription was carried out in a thermocycler (10 min at 25°C; 30 min at 48°C and 5 min at 95°C). The cDNA was subsequently quantified by means of quantitative PCR using the SYBR Green PCR Master Mix (Perkin Elmer), with this determination being carried out in triplicate (in each case using 2-5 OAS primers (mOAS primer 1 CCTTCCTCAA CAGATTCAGA AGGA (SEQ ID No. 17) and mOAS primer 2: TGATCAGACT TTGTCAGACA GAACCT (SEQ ID No. 18)) and GAPDH primers). With a total volume of 57 µl, the stock solution for each triplet contained 37.5 µl of 2 × SYBR Master Mix, 0.75 µl of AmpErase UNG (1 U/µl) and 18.75 µl of DEPC-treated water. For each triplicate determination, 1.5 µl of the forward primer and 1.5 µl of the backward primer were in each case added, in a previously optimized concentration ratio, to 57 µl of the stock solution. In each case 60 µl of the stock solution/primer mixture were mixed with 15 µl of cDNA solution (2 ng/µl) and aliquoted into 3 reaction

tubes. In parallel with this, a stock solution containing primers for determining GAPDH (GAPDH primer 1: ATCAACGGGA AGCCCATCA (SEQ ID No. 15) and GAPDH primer 2: GACATACTCA GCACCGGCCT (SEQ ID No. 16)) was prepared as a reference, then mixed with a further 15 µl of the same cDNA solution and aliquoted into 3 reaction tubes. In addition to this, various cDNA solutions were prepared as a dilution series (4 ng/µl; 2 ng/µl; 1 ng/µl; 0.5 ng/µl and 0.25 ng/µl) in order to determine a standard curve for the GAPDH PCR. In each case, 15 µl of these cDNA solutions were mixed with 60 µl of stock solution/primer mixture for determining GAPDH and aliquoted into 3 reaction tubes. A standard curve for the 2-5 OAS PCR was also established in each case; the same dilutions were used for this standard curve as were also used for the GAPDH standard curve. A PCR mixture without cDNA served as a control. In each case, 15 µl of DEPC water were added to in each case 60 µl of stock solution/primer mixture for determining 2-5 OAS and GAPDH mRNA, respectively, and, after mixing, these solutions were then in each case aliquoted into 3 reaction tubes. The mixtures were amplified in a GeneAmp 5700 (2 min at 50°C; 10 min at 95°C, followed by 3 cycles of 15 s at 96°C and 2 min at 60°C; after that 37 cycles of 15 s at 95°C and 1 min at 60°C). The evaluation was effected by determining the relative abundance of the 2-5 OAS gene in relation to the GAPDH reference. For this, a standard curve was first of all established by plotting the C_T values of the dilution series against the logarithm of the cDNA quantity in the PCR mixture (in ng of transcribed RNA) and the slope (s) of the straight lines was determined. The efficiency (E) of the PCR is then obtained as follows: $E = 10^{-1/s}-1$. The relative abundance (X) of the investigated 2-5 OAS cDNA species (Y) in relation to GAPDH is then: $X=(1+E_{GAPDH})^{C_T(GAPDH)}/(1+E_Y)^{C_T(Y)}$. The numerical values were then standardized by setting the quantity of cDNA from the intact skin obtained from the 10-week-old BALB/c control animals, or from the C57BL/Ks control animals, respectively, equal to 1.

The results of the experiment are shown in table 2. In the first place, it was possible to verify that the expression of 2-5 OAS 1 was markedly higher in the

wounds treated with the glucocorticoid dexamethasone than it was in the normally healing wounds of both 10-week-old and also young and old animals. In addition, expression was observed to be significantly increased in all wounds as compared with the expression in the respective intact skin. This proves that wound healing is accompanied by an increase in the expression of 2-5 OAS. Therefore a regulated expression of OAS and/or its binding partner RNase L is essential for normal progression of wound healing. On the other hand, it was only possible to measure a very weak increase in wound expression, as compared with intact skin, in the poorly healing wounds of diabetic animals whereas it was possible to observe a marked increase in this expression in the control animals. This shows that the expression of 2-5 OAS in diabetic animals exhibiting poor wound healing is incorrectly regulated to a marked degree and that 2-5 OAS and/or its effector Rnase L are particularly preferred for the treatment of venous ulcers and diabetes-associated poorly healing wounds whereby the increase of 2-5 OAS and/or its effector Rnase L expression and/or activity is a particularly preferred embodiment of the present invention.

Example 3: Using TaqMan analysis to analyze the kinetics of the expression of 2-5 OAS 1 during wound healing in the mouse

The kinetics of the regulation of the expression of 2-5 OAS 1 during normal wound healing in the mouse was investigated by TaqMan analysis in a GeneAmp5700 supplied by Applied Biosystems. Biopsies of normally healing day 1 wounds and intact skin were obtained from 6 untreated, 10-week-old BALB/c mice by cutting with scissors as described in example 1. The isolation of the RNA and the subsequent TaqMan analysis were carried out as described in the previous example. The numerical values were subsequently standardized by setting the quantity of cDNA from the intact skin of the 10-week-old BALB/c control animals equal to 1. The relative changes observed in expression of the murine 2-5 OAS 1 gene during wound healing are compiled in table 3. An increase in expression was observed within 24 h after wounding, which persisted

up to 5 days after wounding. After that, the quantity of 2-5 OAS 1 mRNA in the wound decreased. This experiment indicates that differential expression is essential over a long period of the wound healing. Disturbances in the expression and/or activity of OAS and/or its effector RNAseL can therefore lead to severe disturbances in healing.

Example 4: Differential expression of the human 2-5 OAS-1 gene in human wounds

The aim was now to investigate whether, on the basis of the normally healing wound, the differential regulation, of the expression of the wound-relevant 2-5 OAS gene, which was verified in examples 2 and 3, , could also be observed in humans. To do this, 4 mm biopsies of intact skin were taken, as described above, from 6 patients, while 6 mm biopsies were taken after the time points T=1 h, 1 d, 5 d and 14 d. The biopsies taken at each time point were pooled and the mRNA was isolated as described in the previous example. After that, quantification was carried out by means of TaqMan analysis, as described above, however, the abundance of the human 2-5 OAS 1 mRNA was determined relative to cyclophilin in this experiment (EMBL: Y00052). The primers employed for this experiment are: cyclophilin primer 1: ATTGCTGACTGTGGACAACTCG (SEQ ID No. 23), cyclophilin primer 2: AGAAGGAATGATCTGGTGGTTAAGA (SEQ ID No. 24), hOAS primer 1: TCTCAGAAAT ACCCCAGCCA AA (SEQ ID No. 21) and hOAS primer 2: GATGATGTCA ATGGCATGGT TG (SEQ ID No. 22). The evaluation of the experiment is shown in table 4. The results show that hOAS1 expression also strongly increases in human wounds, with this increase in expression lasting up to 14 days after the wounding; i.e. the observation period. This experiment consequently proves that differential regulation of 2-5 OAS in mammalian wounds is essential for the course of wound healing and that the expression and/or activity of 2-5 OAS and/or its effector RNAseL is preferably increased during the whole time course of the healing process for prevention and/or treatment in association with wound healing and/or

its pathological disturbances, particularly venous ulcers and diabetes- associated poorly healing wounds.

Example 5: Differential expression of human 2-5 OAS 1 in human ulcers

In order to demonstrate that the 2'-5'-oligoadenylate synthetase, which has been identified as being relevant to wounds and skin diseases, is not only differentially regulated in humans in normally proceeding wound healing but is also deregulated in disturbed wound healing, biopsies of intact skin, of the wound ground and of the wound edge were taken at the same time point from patients suffering from chronic venous ulcers (*ulcera venosa*) and examined for expression of 2-5 OAS 1. The biopsies taken from in each case 6 test subjects were pooled in the case of each group (intact skin, wound edge and wound ground). cDNAs which were relevant to wound healing were also quantified as described in example 4 using the same primer combinations as in example 4 for determining the amount of 2-5 Oas in venous ulcers. The results of the experiment are summarized in table 5. Slightly diminished expression, as compared with intact skin, was measured at the wound edge of the venous ulcer, the equivalent of the hyperproliferative epithelium of normally healing wounds which forms 1 day after wounding, whereas a marked increase in the expression of 2-5 OAS-1, as compared with that in intact skin, was detected in normally healing day 1 wounds (table 4). No increase in the quantity of 2-5 OAS mRNA was observed in the wound ground of venous ulcers, either. The disregulation of 2-5 OAS is even more pronounced in chronic diabetic ulcers. As reference gene for determining the amount of 2-5 OAS within chronic diabetic ulcers, cyclophilin A was used with the primer combination according to SEQ ID Nr. 25 (Cyclophilin- Primer 3: 5' GGAATGGCAAGACCAGCAAG 3') and SEQ ID Nr. 26 (Cylophilin- Primer4: 5'GGATACTGCGAGCAAATGGG 3'). For determining the amounts of 2-5 OAS the primer combination according to SEQ ID Nr. 21 and SEQ ID Nr. 22 was used. The results clearly show that the 2-5 OAS expression is decreased by factor 1.3 within the proliferative wound edge of chronic diabetic ulcers compared to

normal intact skin of diabetic patients, whereas the wound ground lacks any expression of 2-5 OAS.

The preceding experiments show that a differential up-regulation of 2-5 OAS and/or its effector RNase L is the prerequisite for a normal wound healing process. Poorly healing wounds are incapable of performing this up-regulation and it is this disregulation which leads to severe wound healing disorders, particularly to venous ulcers and to diabetes- associated poorly healing wounds. Thus, the prevention and/or treatment of wound healing disorders, particularly venous ulcers and diabetes- associated poorly healing wounds is accomplished by increasing the expression and/or activity of 2-5 OAS and/or its effector Rnase L.

The results consequently also prove that 2-5 OAS and/or its effector RNAseL can be used for diagnosis in association with wound healing or its disturbances, particularly venous ulcers and diabetes- associated poorly healing wounds.

It will be apparent to those skilled in the art that various modifications can be made to the compositions and processes of this invention. Thus, it is intended that the present invention cover such modifications and variations, provided they come within the scope of the appended claims and their equivalents.

Table 1

	Name	Organism	Protein SEQ ID No.	Access number, protein	cDNA SEQ ID No.	Access number, cDNA
1.	2'-5'-Oligo-adenylate synthetase 1A	*Mus musculus*	1	SWISSPROT: P11928	5	EMBL: X04958
2.	OAS L	*Mus musculus*	2	SWISSPROT: Q9Z2F2	6	EMBL: AF068835
3.	2'-5'-Oligo-adenylate synthetase 1	*Homo sapiens*	3	SWISSPROT: P00973	7	EMBL: X04371
4.	OAS2	*Homo sapiens*	4	SWISSPROT: P29728	8	EMBL: M87434
5.	OAS3	*Homo sapiens*	9	SWISSPROT: Q9Y6K5		EMBL: AF063613
6.	OAS L	*Homo sapiens*	10	SWISSPROT: Q15646		
7	RNAseL	*Mus musculus*	11	trEMBL: Q9ERU7	13	EMBL: AF281045
8.	RNAseL	*Homo sapiens*	12	SWISSPROT: Q05823	14	EMBL: L10381

EMBL: EMBL database

trembl: translated EMBL database

SWISSPROT: SwissProt database

Table 2

Tissue sample	Rel. quantity of 2-5 OAS mRNA
Intact skin, Balb/c control animals	1.00
day 1 Wound, Balb/c control animals	4.67
Intact skin, DEX animals	0.52
day 1 Wound, DEX animals	10.10
Intact skin, young animals	1.24
day 1 Wound, young animals	5.20
Intact skin, old animals	0.62
day 1 Wound, old animals	2.00
Intact skin, C57Bl/Ks control animals	1.00
day 1 Wound, C57Bl/Ks control animals	2.20
Intact skin, diabetic animals	1.01
day 1 Wound, diabetic animals	1.33

Table 3

Time after wounding of Balb/c mice	Rel. quantity of 2-5 OAS mRNA
Intact skin	1.00
1 h	1.37
7 h	1.39
15 h	1.6
24 h	2.02
3 d	2.24
5 d	2.37
7 d	1.33
14 d	0.70

Table 4

	Time after wounding				
	Intact skin	**Wound, 1 h**	**Wound, 24 h**	**Wound, 5 d**	**Wound, 14 d**
Rel. expression of OAS 1 in human biopsies as compared with cyclophilin	1.00	0.48	3.51	1.75	3.14

Table 5

	Intact skin Ulcer patients	wound edge Ulcer patients	wound ground Ulcer patients
Rel. expression of OAS 1 in human biopsies as compared with cyclophilin	1.00	0.85	1.04

Patent Claims

1. Use of at least one 2'-5'-oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10, and/or RNAseL polypeptides according to SEQ ID No. 11 to SEQ ID No. 12, or functional variants thereof, and/or nucleic acids encoding them, or variants thereof, or of a cell which is expressing a 2'-5'-oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10 and/or RNAseL polypeptide according to SEQ ID No. 11 to SEQ ID No. 12 or functional variants thereof and/or nucleic acids encoding them, for diagnosis, prevention and/or treatment of wound healing and/or its pathological disorders.

2. Use according to claim 1, wherein the wound healing concerns a wound which is caused by mechanical, thermic, chemic, or actinic force.

3. Use according to claim 1, wherein the disorder is characterized by a deficiency of 2'-5'-oligoadenylate synthetase mRNA.

4. Use according to claim 1 or 3, wherein the pathological disorder of wound healing is an ulcer of the skin.

5. Use according to claim 4 wherein the ulcer is a diabetes-associated ulcer and/or a venous ulcer.

6. Use according to at least one of claims 1 to 5, wherein the polypeptide is employed in the form of a fusion protein.

7. Use according to at least one of claims 1 to 5, wherein the nucleic acid is employed in the form of an expression vector, a knock-out gene construct or a vector which is applicable in gene therapy.

8. Use according to at least one of claims 1 to 7, wherein the cell is an autologous or a heterologous cell.

9. Use according to claim 8, wherein the cell is a skin cell.

10. Use of an antibody or an antibody fragment, preferably a polyclonal or a monoclonal antibody or antibody fragment, for analysis, diagnosis, prevention and/or treatment of wound healing and/or its pathological disorders, wherein an antibody-producing organism is immunized with a 2'-5'-oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10, and/or RNAseL polypeptides according to SEQ ID No. 11 to SEQ ID No. 12, or functional variants thereof.

11. Use of at least a 2'-5'-oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10, and/or RNAseL polypeptides according to SEQ ID No. 11 to SEQ ID No. 12, or functional variants thereof, and/or nucleic acids encoding them, or variants thereof, or of a cell which is expressing a 2'-5'-oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10 and/or RNAseL polypeptide according to SEQ ID No. 11 to SEQ ID No. 12 or functional variants thereof and/or nucleic acids encoding them, or of an antibody or antibody fragment which is directed the 2'-5'-oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10, and/or RNAseL polypeptides according to SEQ ID No. 11 to SEQ ID No. 12, or functional variants thereof, optionally combined or together with suitable additives and auxiliaries, for identifying pharmacologically active substances in association with wound healing and/or its pathological disorders.

12. Use according to claim 11, wherein the pharmacologically active substances exert an influence on the activity of an oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10, and/or a RNAseL polypeptides according to SEQ ID No. 11 to SEQ ID No. 12, or a functional variant thereof.

13. Use according to claim 11, wherein the pharmacologically active substances exert an influence on the expression of at least one nucleic acid coding for a 2'-5'-oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10, and/or for a RNAseL polypeptides according to SEQ ID No. 11 to SEQ ID No. 12, or for a functional variant thereof.

14. Use according to at least one of claims 1 to 6, wherein at least one oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10, and/or a RNAseL polypeptides according to SEQ ID No. 11 to SEQ ID No. 12, or a functional variant thereof is bound to a solid phase, for diagnosis.

15. Use according to claim 11 or 12, wherein at least one oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10, and/or a RNAseL polypeptides according to SEQ ID No. 11 to SEQ ID No. 12, or a functional variant thereof is bound to a solid phase.

16. Use according to at least one of the claims 11, 13, and 14 wherein at least one oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10, and/or at least one RNAseL polypeptides according to SEQ ID No. 11 to SEQ ID No. 12, or a functional variant thereof, or at least one nucleic acid encoding them, or at least one cell expressing at least one oligoadenylate synthetase polypeptide

according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10, and/or a RNAseL polypeptides according to SEQ ID No. 11 to SEQ ID No. 12, or for a functional variant thereof or a nucleic acid encoding them, or of at least one antibody directed against a 2'-5'-oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10, and/or against a RNAseL polypeptides according to SEQ ID No. 11 to SEQ ID No. 12, or functional variants thereof, is fixed on a support material, for the production of an array for the analysis in association with wound healing and/or its pathological disorders.

17. Use according to at least one of claims 1 to 5, wherein the at least one nucleic acid coding for a 2'-5'-oligoadenylate synthetase polypeptide according to SEQ ID No. 1 to SEQ ID No. 4 or SEQ ID No. 9 to SEQ ID No. 10, and/or a RNAseL polypeptides according to SEQ ID No. 11 to SEQ ID No. 12, is used in the form of a probe or a primer.

18. Use according to claim 18, wherein the probe is a DNA or a RNA.

19. Use according to claim 18, wherein the primer is a DNA or a RNA.

SEQUENCE LISTING

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<120> Use of polypeptides, or nucleic acids encoding them, of a 2'-5'-oligoadenylate synthetase and/or RNAseL for diagnosis, prevention or treating of wound healing, and their use for identifying pharmacologically active substances

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Gly Gln Leu Thr Gly Ser Tyr Lys Pro Asn Pro Gln Ile 169

Tyr Val Lys Leu Ile Glu Glu Cys Thr Asp Leu Gln Lys 182

Glu Gly Glu Phe Ser Thr Cys Phe Thr Glu Leu Gln Arg 195

Asp Phe Leu Lys Gln Arg Pro Thr Lys Leu Lys Ser Leu 208

Ile Arg Leu Val Lys His Trp Tyr Gln Asn Cys Lys Lys 221

Lys Leu Gly Lys Leu Pro Pro Gln Tyr Ala Leu Glu Leu 234

Leu Thr Val Tyr Ala Trp Glu Arg Gly Ser Met Lys Thr 247

His Phe Asn Thr Ala Gln Gly Phe Arg Thr Val Leu Glu 260

Leu Val Ile Asn Tyr Gln Gln Leu Cys Ile Tyr Trp Thr 273

Lys Tyr Tyr Asp Phe Lys Asn Pro Ile Ile Glu Lys Tyr 286

Leu Arg Arg Gln Leu Thr Lys Pro Arg Pro Val Ile Leu 299

Asp Pro Ala Asp Pro Thr Gly Asn Leu Gly Gly Gly Asp 312

Pro Lys Gly Trp Arg Gln Leu Ala Gln Glu Ala Glu Ala 325

Trp Leu Asn Tyr Pro Cys Phe Lys Asn Trp Asp Gly Ser 338

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Ser Gln Arg Asp Ile Leu Asp Lys Thr Gly Asp Lys Leu 104
Lys Phe Cys Leu Phe Thr Lys Trp Leu Lys Asn Asn Phe 117
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Trp Ile Tyr Arg Glu Leu Lys Arg Ser Leu Asp Lys Thr 169
Asn Ala Ser Pro Gly Glu Phe Ala Val Cys Phe Thr Glu 182
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Lys Asp Leu Ile Leu Leu Ile Lys His Trp His Gln Gln 208
Cys Gln Lys Lys Ile Lys Asp Leu Pro Ser Leu Ser Pro 221
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Gly Cys Arg Lys Asp Asn Phe Asp Ile Ala Glu Gly Val 247
Arg Thr Val Leu Glu Leu Ile Lys Cys Gln Glu Lys Leu 260
Cys Ile Tyr Trp Met Val Asn Tyr Asn Phe Glu Asp Glu 273
Thr Ile Arg Asn Ile Leu Leu His Gln Leu Gln Ser Ala 286
Arg Pro Val Ile Leu Asp Pro Val Asp Pro Thr Asn Asn 299

Val Ser Gly Asp Lys Ile Cys Trp Gln Trp Leu Lys Lys 312

Glu Ala Gln Thr Trp Leu Thr Ser Pro Asn Leu Asp Asn 325

Glu Leu Pro Ala Pro Ser Trp Asn Val Leu Pro Ala Pro 338

Leu Phe Thr Thr Pro Gly His Leu Leu Asp Lys Phe Ile 351

Lys Glu Phe Leu Gln Pro Asn Lys Cys Phe Leu Glu Gln 364

Ile Asp Ser Ala Val Asn Ile Ile Arg Thr Phe Leu Lys 377

Glu Asn Cys Phe Arg Gln Ser Thr Ala Lys Ile Gln Ile 390

Val Arg Gly Gly Ser Thr Ala Lys Gly Thr Ala Leu Lys 403

Thr Gly Ser Asp Ala Asp Leu Val Val Phe His Asn Ser 416

Leu Lys Ser Tyr Thr Ser Gln Lys Asn Glu Arg His Lys 429

Ile Val Lys Glu Ile His Glu Gln Leu Lys Ala Phe Trp 442

Arg Glu Lys Glu Glu Glu Leu Glu Val Ser Phe Glu Pro 455

Pro Lys Trp Lys Ala Pro Arg Val Leu Ser Phe Ser Leu 468

Lys Ser Lys Val Leu Asn Glu Ser Val Ser Phe Asp Val 481

Leu Pro Ala Phe Asn Ala Leu Gly Gln Leu Ser Ser Gly 494

Ser Thr Pro Ser Pro Glu Val Tyr Ala Gly Leu Ile Asp 507

Leu Tyr Lys Ser Ser Asp Leu Pro Gly Gly Glu Phe Ser 520

Thr Cys Phe Thr Val Leu Gln Arg Asn Phe Ile Arg Ser 533

Arg Pro Thr Lys Leu Lys Asp Leu Ile Arg Leu Val Lys 546

His Trp Tyr Lys Glu Cys Glu Arg Lys Leu Lys Pro Lys 559

Gly Ser Leu Pro Pro Lys Tyr Ala Leu Glu Leu Leu Thr 572

Ile Tyr Ala Trp Glu Gln Gly Ser Gly Val Pro Asp Phe 585

Asp Thr Ala Glu Gly Phe Arg Thr Val Leu Glu Leu Val 598

Thr Gln Tyr Gln Gln Leu Gly Ile Phe Trp Lys Val Asn 611

Tyr Asn Phe Glu Asp Glu Thr Val Arg Lys Phe Leu Leu 624

Ser Gln Leu Gln Lys Thr Arg Pro Val Ile Leu Asp Pro 637

Gly Glu Pro Thr Gly Asp Val Gly Gly Gly Asp Arg Trp 650

Cys Trp His Leu Leu Asp Lys Glu Ala Lys Val Arg Leu 663

Ser Ser Pro Cys Phe Lys Asp Gly Thr Gly Asn Pro Ile 676

Pro Pro Trp Lys Val Pro Thr Met Gln Thr Pro Gly Ser 689

Cys Gly Ala Arg Ile His Pro Ile Val Asn Glu Met Phe 702

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Gly Thr Ala Leu Lys Gly Gly Cys Asp Ser Glu Leu Val 78

Ile Phe Leu Asp Cys Phe Lys Ser Tyr Val Asp Gln Arg 91

Ala Arg Arg Ala Glu Ile Leu Ser Glu Met Arg Ala Ser 104

Leu Glu Ser Trp Trp Gln Asn Pro Val Pro Gly Leu Arg 117

Leu Thr Phe Pro Glu Gln Ser Val Pro Gly Ala Leu Gln 130

Phe Arg Leu Thr Ser Val Asp Leu Glu Asp Trp Met Asp 143

Val Ser Leu Val Pro Ala Phe Asn Val Leu Gly Gln Ala 156

Gly Ser Ala Val Lys Pro Lys Pro Gln Val Tyr Ser Thr 169

Leu Leu Asn Ser Gly Cys Gln Gly Gly Glu His Ala Ala 182

Cys Phe Thr Glu Leu Arg Arg Asn Phe Val Asn Ile Arg 19

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Thr Leu Pro Pro Val Tyr Ala Leu Glu Leu Leu Thr Ile 234

Phe Ala Trp Glu Gln Gly Cys Lys Lys Asp Ala Phe Ser 247

Leu Gly Glu Gly Leu Arg Thr Val Leu Gly Leu Ile Gln 260

Gln His Gln His Leu Cys Val Phe Trp Thr Val Asn Tyr 273

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His Val Lys Arg Pro Arg Pro Val Ile Leu Asp Pro Ala 299
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Trp Asp Leu His Ala Gln Glu Ala Ala Ser Cys Tyr Asp 325
His Pro Cys Phe Leu Arg Gly Met Gly Asp Pro Val Gln 338
Ser Trp Lys Gly Pro Gly Leu Pro Arg Ala Gly Cys Ser 351
Gly Leu Gly His Pro Ile Gln Leu Asp Pro Asn Gln Lys 364
Thr Pro Glu Asn Ser Lys Ser Leu Asn Ala Val Tyr Pro 377
Arg Ala Gly Ser Lys Pro Pro Ser Cys Pro Ala Pro Gly 390
Pro Thr Ala Glu Pro Ala Ser Tyr Pro Ser Val Pro Gly 403
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Phe Gln Glu Gln Val Lys Lys Ala Ile Asp Ile Ile Leu 442
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Val Ser Lys Gly Gly Ser Phe Gly Arg Gly Thr Asp Leu 468
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Cys Phe Thr Asp Tyr Lys Asp Gln Gly Pro Arg Arg Ala 494
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Lys Pro Asn Pro Gln Val Tyr Ser Arg Leu Leu Thr Ser 572
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Lys Asn Leu Ile Leu Leu Val Lys His Trp Tyr Arg Gln 611
Val Ala Ala Gln Asn Lys Gly Lys Gly Pro Ala Pro Ala 624
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Cys Ser Phe Leu Lys Glu Asn Cys Phe Arg Asn Ser Pro 793

Ile Lys Val Ile Lys Val Val Lys Gly Gly Ser Ser Ala 806

Lys Gly Thr Ala Leu Arg Gly Arg Ser Asp Ala Asp Leu 819

Val Val Phe Leu Ser Cys Phe Ser Gln Phe Thr Glu Gln 832

Gly Asn Lys Arg Ala Glu Ile Ile Ser Glu Ile Arg Ala 845

Gln Leu Glu Ala Cys Gln Gln Glu Arg Gln Phe Glu Val 858

Lys Phe Glu Val Ser Lys Trp Glu Asn Pro Arg Val Leu 871

Ser Phe Ser Leu Thr Ser Gln Thr Met Leu Asp Gln Ser 884

Val Asp Phe Asp Val Leu Pro Ala Phe Asp Ala Leu Gly 897

Gln Leu Val Ser Gly Ser Arg Pro Ser Ser Gln Val Tyr 910

Val Asp Leu Ile His Ser Tyr Ser Asn Ala Gly Glu Tyr 923

Ser Thr Cys Phe Thr Glu Leu Gln Arg Asp Phe Ile Ile 936

Ser Arg Pro Thr Lys Leu Lys Ser Leu Ile Arg Leu Val 949

Lys His Trp Tyr Gln Gln Cys Thr Lys Ile Ser Lys Gly 962

Arg Gly Ser Leu Pro Pro Gln His Gly Leu Glu Leu Leu 975

Thr Val Tyr Ala Trp Glu Gln Gly Gly Lys Asp Ser Gln 988

Phe Asn Met Ala Glu Gly Phe Arg Thr Val Leu Glu Leu 1001

Val Thr Gln Tyr Arg Gln Leu Cys Ile Tyr Trp Thr Ile 1014

Asn Tyr Asn Ala Lys Asp Lys Thr Val Gly Asp Phe Leu 1027

Lys Gln Gln Leu Gln Lys Pro Arg Pro Ile Ile Leu Asp 1040

Pro Ala Asp Pro Thr Gly Asn Leu Gly His Asn Ala Arg 1053

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Arg Gly Leu Asp Gln Asp Val Arg Val Leu Lys Val Val 65
Lys Val Gly Ser Phe Gly Asn Gly Thr Val Leu Arg Ser 78
Thr Arg Glu Val Glu Leu Val Ala Phe Leu Ser Cys Phe 91
His Ser Phe Gln Glu Ala Ala Lys His His Lys Asp Val 104
Leu Arg Leu Ile Trp Lys Thr Met Trp Gln Ser Gln Asp 117
Leu Leu Asp Leu Gly Leu Glu Asp Leu Arg Met Glu Gln 130
Arg Val Pro Asp Ala Leu Val Phe Thr Ile Gln Thr Arg 143
Gly Thr Ala Glu Pro Ile Thr Val Thr Ile Val Pro Ala 156
Tyr Arg Ala Leu Gly Pro Ser Leu Pro Asn Ser Gln Pro 169
Pro Pro Glu Val Tyr Val Ser Leu Ile Lys Ala Cys Gly 182
Gly Pro Gly Asn Phe Cys Pro Ser Phe Ser Glu Leu Gln 195
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Lys Ala Arg Ser Pro Arg Ala Asn Leu Pro Pro Leu Tyr 234
Ala Leu Glu Leu Leu Thr Ile Tyr Ala Trp Glu Met Gly 247
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Thr Thr Val Met Asp Leu Leu Leu Glu Tyr Glu Val Ile 273
Cys Ile Tyr Trp Thr Lys Tyr Tyr Thr Leu His Asn Ala 286
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Val Ala Glu Gly Tyr Arg Trp Asp Ile Val Ala Gln Arg 325
Ala Ser Gln Cys Leu Lys Gln Asp Cys Cys Tyr Asp Asn 338
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Asp Phe Asn Leu Ile Val Asn Pro Tyr Glu Pro Ile Arg 377
Lys Val Lys Glu Lys Ile Arg Arg Thr Arg Gly Tyr Ser 390
Gly Leu Gln Arg Leu Ser Phe Gln Val Pro Gly Ser Glu 403
Arg Gln Leu Leu Ser Ser Arg Cys Ser Leu Ala Lys Tyr 416
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Ile Pro Ser Glu Ile Gln Val Phe Val Lys Asn Pro Asp 442

Gly Gly Ser Tyr Ala Tyr Ala Ile Asn Pro Asn Ser Phe 455
Ile Leu Gly Leu Lys Gln Gln Ile Glu Asp Gln Gln Gly 468
Leu Pro Lys Lys Gln Gln Gln Leu Glu Phe Gln Gly Gln 481
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Ala Cys Glu Asp Thr Trp Gly Trp Thr Pro Leu His Asn 65
Ala Val Gln Ala Gly Arg Val Asp Ile Val Asn Leu Leu 78
Leu Ser His Gly Ala Asp Pro His Arg Arg Lys Lys Asn 91
Gly Ala Thr Pro Phe Ile Ile Ala Gly Ile Gln Gly Asp 104
Val Lys Leu Leu Glu Ile Leu Leu Ser Cys Gly Ala Asp 117
Val Asn Glu Cys Asp Glu Asn Gly Phe Thr Ala Phe Met 130
Glu Ala Ala Glu Arg Gly Asn Ala Glu Ala Leu Arg Phe 143
Leu Phe Ala Lys Gly Ala Asn Val Asn Leu Arg Arg Gln 156
Thr Thr Lys Asp Lys Arg Arg Leu Lys Gln Gly Gly Ala 169
Thr Ala Leu Met Ser Ala Ala Glu Lys Gly His Leu Glu 182
Val Leu Arg Ile Leu Leu Asn Asp Met Lys Ala Glu Val 195
Asp Ala Arg Asp Asn Met Gly Arg Asn Ala Leu Ile Arg 208
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Thr Ser Ile Leu Ile Gln His Gly Ala Asp Val Asn Val 234

Arg Gly Glu Arg Gly Lys Thr Pro Leu Ile Ala Ala Val 247

Glu Arg Lys His Thr Gly Leu Val Gln Met Leu Leu Ser 260

Arg Glu Gly Ile Asn Ile Asp Ala Arg Asp Asn Glu Gly 273

Lys Thr Ala Leu Leu Ile Ala Val Asp Lys Gln Leu Lys 286

Glu Ile Val Gln Leu Leu Leu Glu Lys Gly Ala Asp Lys 299

Cys Asp Asp Leu Val Trp Ile Ala Arg Arg Asn His Asp 312

Tyr His Leu Val Lys Leu Leu Leu Pro Tyr Val Ala Asn 325

Pro Asp Thr Asp Pro Pro Ala Gly Asp Trp Ser Pro His 338

Ser Ser Arg Trp Gly Thr Ala Leu Lys Ser Leu His Ser 351

Met Thr Arg Pro Met Ile Gly Lys Leu Lys Ile Phe Ile 364

His Asp Asp Tyr Lys Ile Ala Gly Thr Ser Glu Gly Ala 377

Val Tyr Leu Gly Ile Tyr Asp Asn Arg Glu Val Ala Val 390

Lys Val Phe Arg Glu Asn Ser Pro Arg Gly Cys Lys Glu 403

Val Ser Cys Leu Arg Asp Cys Gly Asp His Ser Asn Leu 416

Val Ala Phe Tyr Gly Arg Glu Asp Asp Lys Gly Cys Leu 429

Tyr Val Cys Val Ser Leu Cys Glu Trp Thr Leu Glu Glu 442

Phe Leu Arg Leu Pro Arg Glu Glu Pro Val Glu Asn Gly 455

Glu Asp Lys Phe Ala His Ser Ile Leu Leu Ser Ile Phe 468

Glu Gly Val Gln Lys Leu His Leu His Gly Tyr Ser His 481

Gln Asp Leu Gln Pro Gln Asn Ile Leu Ile Asp Ser Lys 494

Lys Ala Val Arg Leu Ala Asp Phe Asp Gln Ser Ile Arg 507

Trp Met Gly Glu Ser Gln Met Val Arg Arg Asp Leu Glu 520

Asp Leu Gly Arg Leu Val Leu Tyr Val Val Met Lys Gly 533

Glu Ile Pro Phe Glu Thr Leu Lys Thr Gln Asn Asp Glu 546

Val Leu Leu Thr Met Ser Pro Asp Glu Glu Thr Lys Asp 559

Leu Ile His Cys Leu Phe Ser Pro Gly Glu Asn Val Lys 572

Asn Cys Leu Val Asp Leu Leu Gly His Pro Phe Phe Trp 585

Thr Trp Glu Asn Arg Tyr Arg Thr Leu Arg Asn Val Gly 598

Asn Glu Ser Asp Ile Lys Val Arg Lys Cys Lys Ser Asp 611

Leu Leu Arg Leu Leu Gln His Gln Thr Leu Glu Pro Pro 624

Arg Ser Phe Asp Gln Trp Thr Ser Lys Ile Asp Lys Asn 637

Val Met Asp Glu Met Asn His Phe Tyr Glu Lys Arg Lys 650

Lys Asn Pro Tyr Gln Asp Thr Val Gly Asp Leu Leu Lys 663

Phe Ile Arg Asn Ile Gly Glu His Ile Asn Glu Glu Lys 676

Lys Arg Gly Met Lys Glu Ile Leu Gly Asp Pro Ser Arg 689

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Phe Gln Glu Glu Glu Gly Gly Trp Thr Pro Leu His Asn 65
Ala Val Gln Met Ser Arg Glu Asp Ile Val Glu Leu Leu 78
Leu Arg His Gly Ala Asp Pro Val Leu Arg Lys Lys Asn 91
Gly Ala Thr Pro Phe Ile Leu Ala Ala Ile Ala Gly Ser 104
Val Lys Leu Leu Lys Leu Phe Leu Ser Lys Gly Ala Asp 117
Val Asn Glu Cys Asp Phe Tyr Gly Phe Thr Ala Phe Met 130
Glu Ala Ala Val Tyr Gly Lys Val Lys Ala Leu Lys Phe 143
Leu Tyr Lys Arg Gly Ala Asn Val Asn Leu Arg Arg Lys 156
Thr Lys Glu Asp Gln Glu Arg Leu Arg Lys Gly Gly Ala 169
Thr Ala Leu Met Asp Ala Ala Glu Lys Gly His Val Glu 182
Val Leu Lys Ile Leu Leu Asp Glu Met Gly Ala Asp Val 195
Asn Ala Cys Asp Asn Met Gly Arg Asn Ala Leu Ile His 208
Ala Leu Leu Ser Ser Asp Asp Ser Asp Val Glu Ala Ile 221
Thr His Leu Leu Leu Asp His Gly Ala Asp Val Asn Val 234
Arg Gly Glu Arg Gly Lys Thr Pro Leu Ile Leu Ala Val 247
Glu Lys Lys His Leu Gly Leu Val Gln Arg Leu Leu Glu 260
Gln Glu His Ile Glu Ile Asn Asp Thr Asp Ser Asp Gly 273

Lys Thr Ala Leu Leu Leu Ala Val Glu Leu Lys Leu Lys 286

Lys Ile Ala Glu Leu Leu Cys Lys Arg Gly Ala Ser Thr 299

Asp Cys Gly Asp Leu Val Met Thr Ala Arg Arg Asn Tyr 312

Asp His Ser Leu Val Lys Val Leu Leu Ser His Gly Ala 325

Lys Glu Asp Phe His Pro Pro Ala Glu Asp Trp Lys Pro 338

Gln Ser Ser His Trp Gly Ala Ala Leu Lys Asp Leu His 351

Arg Ile Tyr Arg Pro Met Ile Gly Lys Leu Lys Phe Phe 364

Ile Asp Glu Lys Tyr Lys Ile Ala Asp Thr Ser Glu Gly 377

Gly Ile Tyr Leu Gly Phe Tyr Glu Lys Gln Glu Val Ala 390

Val Lys Thr Phe Cys Glu Gly Ser Pro Arg Ala Gln Arg 403

Glu Val Ser Cys Leu Gln Ser Ser Arg Glu Asn Ser His 416

Leu Val Thr Phe Tyr Gly Ser Glu Ser His Arg Gly His 429

Leu Phe Val Cys Val Thr Leu Cys Glu Gln Thr Leu Glu 442

Ala Cys Leu Asp Val His Arg Gly Glu Asp Val Glu Asn 455

Glu Glu Asp Glu Phe Ala Arg Asn Val Leu Ser Ser Ile 468

Phe Lys Ala Val Gln Glu Leu His Leu Ser Cys Gly Tyr 481

Thr His Gln Asp Leu Gln Pro Gln Asn Ile Leu Ile Asp 494

Ser Lys Lys Ala Ala His Leu Ala Asp Phe Asp Lys Ser 507

Ile Lys Trp Ala Gly Asp Pro Gln Glu Val Lys Arg Asp 520

Leu Glu Asp Leu Gly Arg Leu Val Leu Tyr Val Val Lys 533

Lys Gly Ser Ile Ser Phe Glu Asp Leu Lys Ala Gln Ser 546

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Lys Asp Leu Ile His Arg Leu Phe His Pro Gly Glu His 572

Val Arg Asp Cys Leu Ser Asp Leu Leu Gly His Pro Phe 585

Phe Trp Thr Trp Glu Ser Arg Tyr Arg Thr Leu Arg Asn 598

Val Gly Asn Glu Ser Asp Ile Lys Thr Arg Lys Ser Glu 611

Ser Glu Ile Leu Arg Leu Leu Gln Pro Gly Pro Ser Glu 624

His Ser Lys Ser Phe Asp Lys Trp Thr Thr Lys Ile Asn 637

Glu Cys Val Met Lys Lys Met Asn Lys Phe Tyr Glu Lys 650

Arg Gly Asn Phe Tyr Gln Asn Thr Val Gly Asp Leu Leu 663

Lys Phe Ile Arg Asn Leu Gly Glu His Ile Asp Glu Glu 676

Lys His Lys Lys Met Lys Leu Lys Ile Gly Asp Pro Ser 689

Leu Tyr Phe Gln Lys Thr Phe Pro Asp Leu Val Ile Tyr 702

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